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Have you ever thought of going into industry?

DOES Britain do enough to ensure that the merits of industry as a career are presented to young people?

In the last year the feeble productivity performance of British industry has been brought to public attention with depressing frequency. The figures speak for themselves: in five years the productivity index has risen by only 13%, the index of actual production by less than 2%. As we have noted before, the academic world has been weighing in recently with its own remedies, generally aimed at improving the rather bad university/industry links at the postgraduate end of the spectrum. Raising the productive capacity of industry has also been the recent theme of Mr Fred Mulley, Secretary of State for Education and Science, who has been urging students to think more of industrial careers and less of academe or the civil service.

There are, of course, two fairly distinct routes by which graduates get into industry. One is by making a definite choice around the age of seventeen to pursue engineering at university. The other is by pursuing some other course at university which does not exclude the possibility of working in industry. But it is by no means obvious that the student of seventeen will have enough information available to him or herself to make a reasoned judgement on whether to go into engineering.

Ask any schoolteacher what causes a child to opt for engineering and the most likely answer is that family influences play a major part. Parents or uncles who are themselves engineers seem to be the examples which lead many young people into the profession themselves. In this respect, engineering runs parallel to farming and medicine. The next most common reason for going into engineering is the conspicuous presence of a potential employer, probably in the local town—though three years later the graduate may well have a different opinion about returning home, of course.

Both of these reasons are rather good ones with a high content of realism in them, but what of the student without engineering in the family and with no particular employment in mind? The claims of engineering are, in general, rather poorly represented to him or her. Quite rightly there is a strong insistence in schools on the pur-

suit of mathematics, physics and chemistry, so few engineers are to be found amongst schoolteachers. Small wonder, then, that many pupils will have never seriously considered engineering as an option to be set against a 'science' course at university.

Obviously those who do choose to do science have a chance to enter industry in a variety of ways upon graduating. But their period at university is likely to be one in which they will hear that pay in industry is not as good as it could be, because industry does not prize its graduates. They will also hear that the prospects of a science-trained employee getting to the boardroom are decidedly inferior to those of, say, an accountant. And even if these statements are not universally true, few in the university science environment are in a position to, nor sometimes wish to, speak in defence of industry.

There are many ways in which the recruitment of scientists for industry and the recruitment of students into engineering deserve attention, but the greatest advance surely awaits the removal of the barriers which in most universities separate engineering students from science students. In many cases hardly a course is taken in common even though the subject matter, particularly in mathematics and physics, runs broadly parallel. If a physics department wishes to teach electronics it will call on a physicist to do so; if an electrical engineering department needs a course in solid state physics it will be taught by an electrical engineer. These tendencies are greatest in the older established universities. The result is often inferior teaching, and, worse, the erection of disciplinary barriers by students themselves.

In recent months there has been some fairly radical talk about the need for postgraduate training modelled on the (best) American institutions. These same institutions are offering undergraduates a diversity which is unmatched in Britain and which allows the student to delay the difficult specialisation decision as long as possible. Critics claim that this leads to academically lightweight graduates: it does on the other hand lead to a greater maturity in making decisions about careers. And who can say that in the United States industry has not profited from the flow of excellent graduates? □



Paul Richards, coordinator of research for the International African Institute's Environmental Review Unit, examines new approaches to environmental research and explains

What environmental crisis means in Africa

ENVIRONMENTAL crisis has become a major neurosis of the seventies, and monitoring and resource management two of its most commonly advocated therapies. But these therapies mean different things in different parts of the world. For richer industrial countries the focus is on the conservation of fossil fuel resources and on pollution problems. But in poorer regions securing the year's food supply from an increasingly overstretched agricultural system frequently overwhelms all other considerations. Atmospheric pollution and petrol rationing are minor irritations in a context of underdevelopment so severe that up to 50% of all live-born children die before they reach the age of five. For the poorest countries, therefore, dead fish and factory-poisoned rivers might be considered welcome evidence of a turn for the better and a small price to pay for escape to prosperity. In contexts such as these, environmental monitoring will tend to focus on nutritional rather than pollution levels, and resource management must, almost of necessity, emphasise quick exploitation rather than longer term conservation.

The distinction between richer and poorer countries in environmental matters goes much deeper than the mere exigencies of current economic planning, however. Environmental research is often location specific, and poorer regions have had less than their fair share of attention in this respect. This in itself is a factor making for continued poverty. Tropical climatology, for example, is a much less well-researched and closely monitored field than its temperate latitude equivalent, with the result that recent drought in Africa has been much less easy to foresee and its consequences much less easy to plan for. But by far and away the most important distinction is that, by taking part in the world trading system, richer nations have been able to diversify and build flexibility into their economies while poorer nations have found themselves beset by a process of structural simplification rendering them less able to withstand environmental shock. Thus the export crop plantation and pesticide spray replace the ecologically subtle polyculture of the subsistence farm with its natural checks and balances. Not only is the new system more vulnerable to pests and diseases, but many plants, skills and habits associated with a sub-

Drought in the Horn of Africa (Photo: Keystone)

sistence way of life disappear irretrievably. Should famine then occur for whatever reason—political or natural—suffering is likely to be much greater.

The subsistence farmer is a natural survivor under conditions of ecological crisis—his answer to the destruction of his crops by grasshopper or locust is to feed on insects for a time. Development pushes in the opposite direction. I have vivid memories of a village meeting on nutritional problems in Nigeria being forced to disband under the bludgeoning of the bulldog amplifier on a van selling patent medicines for an international pharmaceutical concern. When advertising can penetrate—in this way or via radio—to the most isolated rural areas in Africa, then the imported product of modern manufacture enjoys a powerful advantage over local equivalents. Perfectly acceptable local foodstuffs are traded for volumetrically inferior amounts of processed food or totally inappropriate medicines and tonics, and children malnourished on packaged baby foods then become innocent casualties of a world in which the white-coated image of scientific authority on the advertising hoarding is used to undermine confidence in the old ways for the sake of commercial progress.

In Africa the old relations between man and his environment still have much to tell us. The 'alternative technology' movement in industrially advanced societies is struggling to recover some of the self-sufficient technology of an earlier generation.

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In Africa the crops, cultivation practices, crafts and medicines that support a largely self-sufficient way of life are still part of a living tradition, but a major research effort is now required to establish and preserve this knowledge and skill in permanent form. If growing ecological consciousness in science can lead to a greater awareness of and interest in endangered plants and animals, then surely tropical farming systems, pharmacopaeias and technologies deserve at least equal attention. The task is one of trying to encourage, co-ordinate, publish and where necessary to initiate research into aspects of man-land relationships and pre-industrial technology endangered and discriminated against by processes of 'modernization' and economic development in Africa, in the hope that viable 'alternative' strategies of environmental resource management can thereby be identified.

There is, in fact, a fair amount of activity in this sphere in Africa at the present time, but it is a difficult field in which to work for a number of institutional and organisational reasons. Colonially imposed boundaries and *lingua franca* do not follow ecological lines. Scientists in neighbouring countries may be working on comparable environmental problems but fail to benefit from each others' progress for two reasons: firstly, the anglophone/francophone (not to mention lusophone) barrier is even more difficult to transcend in Africa than it

is in Europe, due to lack of language teaching facilities at secondary school, hardly made good by perfunctory courses in French, English and so on for scientists at university level; and secondly, it often appears easier for an African scientist to arrange study leave and research facilities in Europe or North America than in a neighbouring African country. Comparative approaches, in consequence, are rare, and good local case studies tend to remain local in their impact. But above all, the major difficulty lies in the nature of the work, straddling the divide between social and natural science—an unfortunate line of demarcation hallowed not by logic but by usage.

Leaving aside the arid social-antisocial, natural-supernatural science arguments, there are genuine differences of style and method between social and natural scientists—social scientists, for example, are part of what they study and their language leans towards the polemical in the hope of influencing their material for good. Such differences have to be overcome before the two groups can work together effectively. And yet ethnobotany, 'folk' medicine and 'appropriate' technology would be unthinkable without collaboration between, say, biochemists, ecologists, agronomists and engineers on the one hand and linguists, ethnographers and geographers on the other.

The Environmental Review Unit of the International African Institute is one body trying to find its way across these various barriers and obstacles, partly through its bilingual publications programme, which is based on a working relationship between the

Training for the Environment Programme in Dakar and the IAI (the journal *African Environment/Environnement Africain* and the *Special Report* series of monographs and collections of essays are joint ventures, for example), and partly through a research programme which emphasises the involvement of African scientists and scholars in cross-national studies of environmental problems from a comparative and interdisciplinary point of view. The IAI's traditional strength lies in the linguistic and social science fields, but this is being complemented by links with institutions in Africa having strong natural science representation.

Three major research projects are in the course of being established. The first involves the study of nutritional concepts and strategies in subsistence societies and how these are changing, for better or for worse, under the impact of agricultural development. The second is in a way linked, being an attempt by a group of archaeologists, ethnobotanists and ethnographers to investigate the origins as well as the present-day significance of yam cultivation in Africa. The third project involves looking at the agronomic and ecological conceptions of peasant farmers to assess the role they might play in community-based programmes of environmental monitoring and resource management in Africa; it is based on collaboration between IAI research workers in Africa and the Monitoring and Assessment Research Centre of Chelsea College, London, with initial funding from SCOPE, and has resulted in a preliminary report of a Nigerian case study.

The funding of such research, which falls between the natural and the social sciences, poses problems of its own. But with initial results exhibiting great promise for further development along the lines outlined, the hope is that those who normally fund research on either the social or the natural science side will stretch their terms of reference to cover this vital area. The financial resources required are relatively modest but, even so, no single institution or individual can assemble and effectively combine the range of skill and experience required. Inter-disciplinary co-operation rather than intellectual rivalry is the essential pre-requisite. World wide studies by the Disaster Research Unit at the University of Bradford indicate that, while the probability of the occurrence of geophysical and climate disturbances has remained constant over the last hundred years, 'disasters' have doubled.

The conclusion is plain—namely that human communities are increasingly vulnerable to natural hazard, for social rather than natural reasons. Players of the environmental game appear to have two strategies open to them—minimising maximum losses or maximising potential gains. The modern world opts for the latter in the interests of growth and profit. But someone has to pay the price for this aggressive competitiveness, and it is the poor who end up more vulnerable to ecological breakdown than ever before. Good environmental research is difficult to do because it requires a direct reversal of precisely those qualities of individual rivalry and competitiveness that lie at the root of our present environmental predicament. □

US BUDGET

Escaping the 'New Realism'

Colin Norman explains how science and technology fare

WHEN President Ford unveiled his election year Budget last week, he said it reflected "New Realism" in government policy in the United States. The new realism turned out, however, to be old-style conservatism in the shape of proposals for massive increases in military spending, swingeing cuts in government expenditure in fields such as health and social services, and tax relief for individuals and corporations. Many of those proposals will be unpalatable to the democratic-controlled Congress—which must act on them before they become law—and thus, as a guide to what will actually be spent by government departments and agencies in the 1977 fiscal year (which begins on October 1, 1976), Mr Ford's budget

figures should be treated with considerable caution.

Nevertheless, the budget, which consists of thousands of pages of facts and figures, richly laced with promises and rhetoric, is an important statement of the Administration's political thinking; it sets out in fine detail the programmes for which Mr Ford and his Administration will seek congressional approval in the coming months. As far as science and technology are concerned, the Administration's thinking seems to be surprisingly expansive in view of the frugality displayed elsewhere in the budget.

The extensive axe-wielding which resulted in keeping the total budget request within Mr Ford's target of

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Gerald Ford: 'realist'

\$395,000 million for the federal government next year, left most areas of research and development relatively unscathed. In fact, Mr Ford has proposed hefty increases in several areas

Photo: PA

of science and technology, though there are notable exceptions in a few areas, such as biomedical research and space science.

Putting the figures in their best possible light, Dr H. Guyford Stever, the President's science adviser and Director of the National Science Foundation (NSF), noted last week that the budget contains a total of \$23,500 million for research and development and a further \$1,200 million for scientific equipment and facilities. If Congress agrees to the figures, and if all the money is spent, that would amount to an increase of some \$2,200 million (11%) over anticipated expenditures this year. With inflation now reckoned to be about 6 or 7%, the budget proposals would actually result in some real growth in federal support for science and technology.

The increases are particularly unexpected because federal expenditures on research and development fall almost entirely in the portion of the budget which is relatively controllable. More than half the total budget is now spent on such items as pensions and medical payouts for the poor and elderly, which cannot easily be cut, and thus Mr Ford had to look for most of his savings in the relatively controllable section. Using a bit of the rhetoric which is customary on such occasions, Dr Stever told reporters last week that the fact that science and technology fared well in the budget suggests that "this Administration has clearly placed a high priority on research and development for the achievement of national goals".

Two of the Administration's goals—beefing up military technology and securing long-term energy independence for the United States—would swallow up the lion's share of the increases. But another, more surprising trend emerges from the budget pro-

posals. Basic research, federal support for which has dwindled since the late 1960s, would get a monetary shot in the arm, increasing by about 11%. The National Science Foundation's research budget alone is set for an increase of about 20%, and anticipated support for research and development in colleges and universities is set for a 9% boost.

Within that overall budget, the Administration has decided to give its backing to a number of large new science projects. There is money in the budget to begin construction of a \$78 million positron-electron colliding beam device at Stanford University, NASA has been given the green light to begin building a solar spacecraft to study the maximum sunspot activity in 1979–80, and several new energy projects have been proposed.

But there are also bleak spots. Congress and the Administration have not yet been able to agree on the size of the budget for the National Institutes of Health (NIH) for this year, let alone next year, and a lengthy tussle seems inevitable. Ford, in short, wants to trim this year's budget for NIH below what it was in 1975, and he has proposed only a modest increase above the 1975 budget for 1977 (*see later*). Congress on the other hand, wants to increase the NIH budget substantially. The outcome of that tussle will profoundly affect the entire picture for support of research and development by the federal government, particularly in the universities which receive nearly half their total funds for federal research from NIH. Furthermore, NASA has had to defer plans for several key science projects, including the large space telescope, and the Environmental Protection Agency's research and development budget has suffered from the Administration's knife.

Another area which is troubling

many government science agencies is that Mr Ford wants to cut the size of the civil service, which means that although some agencies may get larger research budgets, they will have to administer them with fewer people.

How is Congress likely to treat the science budget? Traditionally, the Administration's budget request is picked over piecemeal by a number of appropriations subcommittees, each of which handles the budget for one or two departments or agencies. Consequently, it has been difficult for Congress to keep track of the size of the total budget, and it has not been easy for Congress to set priorities for some departments in relation to others. But this year, for the first time, Congress has a fully operational budget system to combat those deficiencies. A Budget Committee will establish an overall target figure for the entire federal budget (which may or may not be the same as Mr Ford's target figure), and it will set limits for each individual appropriations subcommittee to stick to. Though the Budget Committee is likely to sympathise with Mr Ford's attempt to reduce the size of the federal deficit—the Administration's budget anticipates that the deficit will shrink from \$76,000 million this year to \$43,000 million next—it will probably attempt to do it in a different way. In short, the major budget battles are likely to centre on the size of the defence budget, with Congress seeking to reduce it below Mr Ford's proposed level, and the size of the budget for the Department of Health, Education and Welfare, which Congress will try to increase. Since those two departments fund the bulk of federal research and development, the outcome of those battles will shape the overall picture for science and technology.

The following are the major highlights in the budget for science and

Table 1 Conduct of research and development of major departments and agencies (in \$ million)

Department or agency	Obligations			Outlays		
	1975 actual	1976 estimate	1977 estimate	1975 actual	1976 estimate	1977 estimate
Defence—Military functions	8,987	9,879	11,198	9,189	9,468	10,762
National Aeronautical and Space Administration	3,088	3,473	3,573	3,181	3,402	3,550
Energy Research and Development Administration	2,071	2,812	3,282	1,862	2,423	3,042
Health, Education, and Welfare	2,395	2,369	2,570	2,108	2,366	2,512
National Science Foundation	604	628	726	571	602	647
Agriculture	424	483	507	418	486	510
Transportation	291	340	319	307	338	304
Interior	296	332	316	265	307	310
Environmental Protection Agency	258	305	241	207	324	298
Commerce	222	247	243	220	239	233
Veterans Administration	99	108	106	97	99	100
Nuclear Regulatory Commission	61	97	109	54	88	103
Housing and Urban Development	57	62	70	52	57	67
Justice	44	65	41	44	50	44
All other	126	138	164	124	142	156
Total	19,023	21,338	23,465	18,699	20,391	22,638
Total, conduct of research	6,759	7,150	7,782	6,355	7,192	7,709
Total, conduct of development	12,264	14,188	15,683	12,344	13,199	14,929

technology, and the likely congressional arguments.

National defence

Claiming that "we dare not do less", Mr Ford has proposed a record-breaking budget for the Department of Defence (DOD), amounting to \$114,000 million, of which some \$101,000 million would actually be spent in the 1977 fiscal year. Consequently, military research and development in DOD is set for a whopping budget increase, from about \$9,900 million to \$11,200 million. In addition, Mr Ford has proposed that the Energy Research and Development Administration (ERDA) should spend another \$775 million on nuclear weapons research. Thus, according to Ford's proposals, military research and development would carry off more than half the total science and technology budget, and its share would actually increase.

Most of that money would be spent on weapons development, with ballistic missile projects receiving highest priority. Defence-related basic research is projected to rise from \$330 million to \$383 million. DOD is also under an edict to reduce its civilian staff by some 26,000 which could have repercussions in defence laboratories, and it could also result in more defence work being performed in colleges and universities.

Congress is unlikely to approve an overall defence budget of that size, however, and a major political battle over several weapons programmes should be anticipated.

Energy research and development

The second area to be favoured with large proposed increases is support of energy research and development programmes, most of which are now clumped in the Energy Research and Development Administration. Mr Ford's budget includes some \$3,200 million for direct energy research and development, together with closely related environmental and basic research. That would amount to an increase of nearly 40%. Congress is not likely to quibble about the fact that the budget for such efforts is increasing by leaps and bounds, but it is likely to question the way in which the money is to be divided between nuclear and non-nuclear technologies.

The Ford budget proposes a sharp increase in spending on fission reactor development and related studies on the fuel cycle, from \$852 million this year to \$1,215 million next. A good deal of that increase would be swallowed up by the Liquid Metal Fast Breeder Reactor (LMFBR) programme, which alone would receive \$655 million next year. That heavy emphasis on the

LMFBR is consistent with a recent policy statement by ERDA administrator Dr Robert C. Seamans Jr, who said that after studying the environmental, economic and technological questions surrounding the breeder programme, he has decided that ERDA should proceed rapidly with LMFBR development. The goal is now to have the first commercial LMFBR in operation by 1993.

The other noteworthy increase in the budget for energy research and development is in the thermonuclear programme. Mr Ford has proposed that the fusion programme should receive a total of \$392 million next year, a massive boost from this year's budget of \$250 million. A large part of the increase is earmarked for the Tokamak fusion test reactor, a major facility under construction at Princeton University which should be in operation by about 1982. The laser fusion programme is also set for a substantial increase, from \$83.6 million to \$101 million.

Large percentage increases have also been proposed for non-nuclear technologies, such as solar energy, development of oil shale, geothermal power, and conversion of coal to liquid and gaseous fuels. But they would continue to receive much less than half the total energy research and development budget.

Congress is likely to question the increased commitment to nuclear energy which the budget entails, but nuclear opponents in Congress probably lack the votes to reduce the nuclear budget substantially. A good deal of noisy skirmishing should be anticipated, however.

Biomedical research

Once again, the big loser in the federal research and development budget seems to be biomedical research. The situation is, however, greatly complicated by the fact that Congress and the Ford Administration have been unable to agree on the size of the budget for the Department of Health, Education and Welfare (HEW)—of which NIH is a part—for this year, and until they do, the outlook for biomedical research support is very uncertain.

Late last year, Congress passed a bill for HEW which contains very large increases in Mr Ford's original budget request for NIH in the 1976 fiscal year, which is already well under way. Mr Ford vetoed the measure, however, because he claimed that it would fuel inflation and instead, he has now proposed that NIH should receive slightly less money this year than it received last. And the budget he has proposed for 1977 would not even restore NIH to the 1975 level if inflation is taken into account. The various budget pro-



Dr H. Guyford Stever, NSF Director

posals for NIH are shown in Table 2.

Congress will try this week to override Mr Ford's veto of the HEW bill. If it is successful, then NIH would receive a hefty budget increase this year. Otherwise, if NIH is funded at the level proposed by Mr Ford, there would be insufficient money available to fund any new projects until next October. In other words, support for new biomedical research would grind completely to a halt. Moreover, NIH budget officials have calculated that Mr Ford's proposed budget for next year would allow them to fund only 37% of the grant applications approved by peer review groups. The outcome of Congress's attempt to override the veto is difficult to predict, but the prevailing opinion on Capitol Hill seems to be that it will be successful.

Nevertheless, Mr Ford's budget proposal for 1977 shows a very significant new trend. For the first time in five years, the Administration has suggested that funds for cancer research should be held in check while support for other areas of biomedical research should be allowed to grow. That suggestion follows months of debate about relative priorities in biomedical research, centred on the fact that cancer research funds have grown by 280% between 1973 and 1975, while NIH's budget for other research has increased by only 20% in the same period.

It is questionable whether Congress will go along with such a redistribution of funds for biomedical research, but at least the proposal would sharpen discussions of research priorities.

Another problem in NIH's proposed budget is that it contains no provision for increases in NIH staff positions. According to Dr Donald Fredrickson, Director of NIH, that restriction could be especially burdensome because NIH is starting up an entirely new institute devoted to study of the ageing process, and it will be forced to do it without any new people.

Congress and the Administration should reach some agreement—or at least a stalemate—on NIH's 1976 budget in the next few weeks. The tussle will then begin over the size of the budget for next year, all of which makes for extremely difficult planning not only at NIH but also in the universities, which now receive about \$1,300 million a year in grants and contracts from NIH.

The National Science Foundation

The bulk of federal funds for basic research come from the National Science Foundation (NSF). Its budget is therefore closely watched in the scientific community as a barometer of federal support for basic research. About 87% of NSF's money is spent on grants to scientists in colleges and universities.

The budget proposed for NSF would result in a total increase of about 11%, reaching \$812 million. Within that total, NSF's basic research programmes would grow by nearly 20%, while its applied research and education support would either decrease or remain constant. According to Dr Stever, the "specific aim" of the proposed increases "is to counteract the gradual decrease in federal support for basic research which has declined by about 23% in terms of constant dollars since 1968".

The proposed increases in research support would be applied more or less evenly throughout the sciences. No new large projects are planned, but the budget contains sufficient money to continue construction on schedule of the very large array telescope system which is being built in New Mexico.

Usually, NSF's budget attracts little attention in Congress, and aside from minor tinkering by the appropriations committees, the Foundation receives close to the amount of money requested. But last year NSF came in for very heavy criticism on Capitol Hill from Congressmen and Senators who believed that it was either supporting trivial research or that it was developing morally unacceptable school science courses. Consequently, Congress cut NSF's budget for this year, and arranged that basic research bore the brunt of the reductions. Close scrutiny of NSF's programmes can be expected again this year, and Stever warned last week that if the decline in basic research support is to be halted, "Congress must rally round this budget".

Space science

Once again, the National Aeronautics and Space Administration (NASA) has been cut back and space science has been squeezed especially hard. Dr James Fletcher, the Administrator of NASA, said last week that the proposals for NASA mean that 1977 "will be another year of tight constraints, minimal budgets and limited new activities".

The chief problem is that NASA's total budget is being held approximately constant, while expenditures on the space shuttle are increasing rapidly. The net result is that the shuttle is soaking up a growing share of the Agency's budget, leaving little to spare for space science. Next year, for example, out of a total of some \$3,676 million proposed for NASA, the space shuttle will account for \$1,288 million.

The effects of the squeeze will be particularly severe in the budget for

the Office of Space Science, which is set to shrink from \$417 to \$379 million. Only one major new start has been proposed for next year—a so-called solar maximum mission, a spacecraft which will study solar flares and other phenomena during the period of peak solar activity in 1979–80. The chief casualty is the Large Space Telescope (LST), an optical telescope which NASA hopes to fly on an early shuttle mission in the early 1980s. The LST has been accorded top priority by the Space Science Board of the National Academy of Sciences, and NASA was hoping to begin developing it this year. A start has now been deferred, however, until at least 1978.

Another high priority space science mission which will have to wait for at least another year is the proposed mission to send an orbiter and probe to Jupiter in 1981. Again, there is no money in the budget for that mission, but NASA officials are hoping to make a start in 1978. But one planned venture has been scrapped entirely—the proposal to send a Mariner spacecraft to Jupiter and then on to Uranus. Because there is no money in NASA's budget to start work on that mission next year, the opportunity to make use of a rare alignment of the outer planets to send a spacecraft past Jupiter and on to Uranus will be lost. NASA officials are hoping, however, to reprogramme an already-approved spacecraft due to be launched next year, so that it will fly close to Uranus in 1985 after it swings past Saturn.

In past years, Congress has not made many substantial changes to NASA's space science budget, and there is no reason to expect any difference this year. □

Table 2 National Institutes of Health (in \$ thousand)

Institute or Division	1975 Actual	1976 Revised President's Budget	Vetoed 1976 bill*	1977 President's Budget	1977 change over 1976 President's Budget
Cancer	691,666	\$687,394	743,564	\$687,670	+ 276
Heart	324,630	304,702	349,059	342,855	+ 38,153
Dental	50,033	48,592	45,794	52,207	+ 3,615
Arthritis	173,514	161,843	175,172	180,837	+ 18,994
Neurology	142,498	135,139	136,546	146,532	+ 11,393
Allergy	119,452	119,136	118,918	135,615	+ 16,479
General Medical Sciences	187,400	167,538	146,461	193,435	+ 25,897
Child Health	142,435	122,174	126,889	129,883	+ 7,709
Ageing	—	16,071	17,526	26,220	+ 10,149
Eye	44,133	44,435	45,565	46,950	+ 2,515
Environmental Health	35,171	34,023	35,915	46,141	+ 12,118
Research Resources	127,200	83,376	129,931	92,342	+ 8,966
Fogarty Center	5,859	5,404	5,705	7,492	+ 2,088
Library	28,850	29,277	29,065	35,234	+ 5,957
Office of the Director	17,326	18,370	17,896	16,234	+ 2,136
Total, Biomedical Research	2,089,897	1,977,474	2,124,006	2,139,647	+ 162,173
Buildings and Facilities	3,000	3,000	54,000	25,400	+ 22,400
Total, NIH	2,092,897	1,980,474	2,178,006	2,165,047	+ 184,573

* Does not include money for training grants, which is included in other columns. Congress has voted a separate budget of \$124 millions for biomedical training in 1976, which would bring the total for biomedical research in the vetoed bill to \$2,300 million.

UK REPROCESSING CONTROVERSY

Suffering Fuels gladly

It is all down to a Cabinet decision. The media have had their field day, the antinuclear lobbies have voiced their objections and the Select Committee on Science and Technology has declined to investigate the issue. If British Nuclear Fuels Limited (BNFL) gets the green light it wants in the near future the few remaining wrinkles will be ironed out of the deal with the Japanese Enrichment and Reprocessing Group, and irradiated nuclear fuel from Japan will be all but on its way to Britain for reprocessing.

Although spent Japanese fuel is already reprocessed in Britain, few people doubt that the Secretary of State for Energy, Mr Anthony Wedgwood Benn, aimed to give this deal, involving the reprocessing of 4,000 tonnes of fuel over the decade from 1979, a wide public airing. The recent, unattributed "leak" to the popular UK press put the issue before a wider audience than was already following its progress in the up-market newspapers. Speculation over Mr Benn's own attitude added zest to the coverage. And when BNFL followed a public debate in Barrow with another in London this month the presence of Mr Benn ensured a glare of publicity that opponents to the deal could not afford to miss.

But the odds have been stacked against them from the start. Mr Benn has promised that the government will weigh the issues raised by the debate in making its final decision. But the economic benefits which the deal offers, the essential role of reprocessing in Britain's future nuclear programme, and the excellent safety record of the British nuclear industry, all point to a decision in favour of BNFL.

Nor is there any chance that this particular deal will be affected by suggestions that nuclear reprocessing be conducted on a non-commercial basis through an international arrangement. The Pugwash symposium to outline plans towards that end is still some months off; even then it may take several years before proposals percolate through to effective executive levels. The suggestion is anyway irrelevant to concerns about the wisdom of importing foreign radioactive material to Britain. The BNFL reprocessing plant at Windscale in Cumbria, where the Japanese will send their fuel, with its established facilities and 25 years of operational experience, makes it a prime choice for internationally supervised reprocessing.

Most critics believe that reprocessing can actually be avoided altogether.

Some have suggested that benign energy resources be developed to replace nuclear energy, a view more widely regarded as unrealistic, in the short-term at least. Those who accept the need for a continuing reliance on nuclear power, the Pugwash group among them, suggest that a switch to the thorium cycle would provide a viable alternative to reprocessing. That, however, ignores Britain's commitment to the fast breeder reactor. The first FBR will be on-stream by the turn of the century and reprocessing will be an essential part of their nuclear fuel cycle. Moreover, only the most optimistic interpretations of known uranium reserves suggest that there are sufficient natural resources to fuel second generation reactors until FBRs come into their own. The shortfall, it is argued, must inevitably come from reprocessing.

Another factor in Britain's decision, inevitably, will be finance. Throughout the final rounds of its publicity campaign, BNFL has made great play of the deals' commercial advantages. Apart from attracting other foreign customers, mostly in western Europe, the state-owned BNFL could add an estimated £600 millions to Britain's import earnings on completion of the deal. Though the finer details are still under negotiation it is certain that a substantial proportion of that amount—probably between £100–200 millions—will come as a downpayment on the signing of the contract. Payment of the remainder will be spread over the ten-year period covered by the deal. With each consignment sent to Windscale the Japanese will pay an agreed proportion of each batch's reprocessing cost, the total to be made up when the reprocessed material is returned to Japan.

There is no danger that the Japanese fuel will remain in Britain indefinitely. Should BNFL fail to develop the vitrification technique which will allow them to classify the reprocessed material for relatively safe transport, they will withdraw from the deal and the Japanese will repossess the fuel untreated. The Japanese Enrichment and Processing Group are apparently satisfied with that condition. BNFL confidently expect that their recent step-up of vitrification research will ensure the viability of the process by the mid-1980s.

The reprocessed material will contain a small but significant amount of plutonium, a key element of nuclear weaponry. But suggestions that in completing the deal Britain will thereby

contravene the Non-Proliferation Treaty are ill-founded. The present signatories unanimously recognise that the present agreement is not intended to ban nuclear trade with outsiders; moreover, Japan is in a position to ratify the treaty at any time. In addition, under a system administered jointly by the International Atomic Energy Agency and Euratom, a series of detailed arrangements, to which the parties involved must accede, provide safeguards for nuclear trade agreements. The safeguards are specifically designed to prevent the clandestine diversion of material to military uses, and allow the IAEA to conduct checks at any time. It was by entering into a similar arrangement that West Germany was recently able to sell a complete nuclear fuel cycle to Brazil. Similar provisions will apply to the reprocessing deal between Japan and Britain.

There is another area of uncertainty relating to the reprocessing itself. Critics are concerned because the Japanese, who have now adopted the American light water reactor, will send oxide fuel, and not magnox fuel, to Britain. Most of the foreign fuel so far reprocessed at Windscale, coming in the main from Latina in Italy and Tokai Mura in Japan, has been magnox from first generation reactors. The reprocessing of oxide fuel poses greater problems because of the higher activity of the waste. BNFL has gained little experience in oxide reprocessing and what they have is not entirely encouraging. The first oxide reprocessing plant is yet to be built at Windscale, though up until 1973 BNFL reprocessed minor amounts of foreign oxide fuels using a head-end adaptation on the existing magnox plant. The operation was discontinued following a minor incident in which a small cloud of radioactive dust escaped during servicing, contaminating workers. Nonetheless, BNFL aim to have two plants operational by the mid-1980s, handling 1,000 tonnes of material annually. Most of it will come from abroad, according to the company's projections, though all but a small percentage will return to the country of origin.

Trades union officials in Cumbria are eager to see the deal go through. For them at least the issues are clear cut. The deal is likely to create an estimated 2,000 jobs in the area—at the sizeable projected cost of some \$900 million. This the Cabinet may also consider relevant in its deliberations: BNFL itself has deployed the jobs argument in support of its view. But this issue aside, few people doubt the final outcome.

Allan Piper

correspondence

Journal guidelines

SIR,—In your issue of November 27, you ask for comments on "Journal guidelines", a discussion which you began in your issue of November 6. You may be interested in publishing the experience with our new journal, *Intervirology*, which is owned and operated by the Virology Section of the International Society for Microbiological Societies.

The journal has 12 sections, each with its own editor and specialised board of reviewers, who are listed in each issue. For the first five volumes, each author selected the appropriate section of the journal and submitted one copy of the paper to the Section Editor and a second copy to a board reviewer of his choice. In this manner, the author was confident that his paper was being judged by persons whom he regarded as authorities in the area of the work being presented. The board member sent his review to the Section Editor, who also reviewed the paper and then made the decision to accept, modify, reject, or seek additional, anonymous review. The final judgement as to publication was made by the Section Editor.

At the Third International Congress for Virology held in Madrid in September, it was decided to modify this procedure. To provide a consistent balance between known and anonymous reviewers, authors are now required to send one copy of their paper to an editorial board member. Essentially, the reviewer acts as the friend in court whom the author respects as an authority in the field. The author also sends two copies to the Section Editor, who reads one copy as before, but who now regularly sends out a copy for anonymous review. As before, reviews are sent to the Section Editor, who continues to make the final judgement on publication.

The editors hope that the current procedure will satisfy authors that they are fairly represented in the decision-making process and at the same time will allow for non-personal critical evaluation before a paper is accepted or rejected.

Yours faithfully,

JOSEPH L. MELNICK
(Editor-in-Chief)

Department of Virology,
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SIR,—It is extraordinarily difficult to prove the functional value of a complex and subtle social convention such as the preservation of the anonymity of editorial referees. What, for example, is the nature of the 'editorial experience' that Dr C. N. Davies claims (December 18) to confirm his opinion that this long-established custom can be abandoned? His experiment with the *Journal of Aerosol Science* is to be commended; but we shall need to know more about the results before accepting his personal opinion that they are convincing.

Until such empirical evidence is available, I cling rather to the conventional wisdom that anonymity protects the referee from the temptation of softening his remarks to avoid causing personal offence. Most scientists are intellectually conscientious, and will exercise their critical faculties fairly on any paper within their competence; they are seldom (if ever, in my experience) malicious or dishonest. But they know that the delicate competitive/cooperative relationship between colleagues in the same 'Invisible College' cannot stand the stresses that would arise if Dr A had to express, in his own name, in writing, his opinion of the earnest efforts of his rival/friend Dr B. Inevitably, punches would be pulled, and fundamental critical issues would be ducked.

Anonymity is better for all concerned: for the referee, who does not have to mix emotional factors into his intellectual judgements; for the editor, who gets a more honest opinion to guide his decisions; for the reader, who gets more reliable and better expressed papers that have been subjected to a higher standard of criticism; and, strangely enough, for the author who, when his mistakes are pointed out, can vent his chagrin harmlessly in the direction of an impersonal critic without falling into the mortal sin of acquiring a supposed enemy.

The cry against 'authoritarianism' and 'elitism' is, of course, populist in that it appeals to the immediate interest of each individual against the social constraints imposed by the community. But the 'anonymous referee' is only oneself, on the other side of the hill, wearing another uniform. Merton and Zuckermann (*Minerva*, 9, 66; 1971) have found no abuse of the referee system by senior scientists—or whoever the bogies are supposed to be. My own

experience is that the one-way mirror of anonymity facilitates a psychological role reversal, from author to referee, which makes better, more humble, more sceptical scientists of us all, and curbs the vanity and pride of those who claim 'authority'.

This, somewhat schematically and cryptically, is the basis of my assertion that it is a populist folly to disclose the names of referees to authors.

Yours faithfully,

JOHN ZIMAN

University of Bristol, UK

Unit proposal

SIR,—We would like to propose a unit for electrophoretic mobility (u) to replace the current practice of specifying it, for example, as follows: $u = -2.45 \times 10^{-5} \text{ cm s}^{-1} \text{ V cm}^{-1}$ (+ for cations, — for anions).

In honour of Arne Tiselius, who has done more than anyone else to advance electrophoretic methodology, we propose the adoption of the Tiselius Unit: $1 \text{ TU} = 10^{-5} \text{ cm s}^{-1} \text{ V cm}^{-1}$ as the unit of the electrophoretic mobility. The above example for electrophoretic mobility will thus be written: $u = -2.45 \text{ TU}$ in the proposed notation.

This recommendation relies on analogy to the adopted Svedberg Unit S for sedimentation coefficients (s): $1 S = 10^{-13} \text{ s}$, where the sedimentation coefficient

$$s = \frac{dr/dt}{\omega^2 r}$$

measures the sedimentation velocity of a particle in a unit centrifugal field, by analogy with the electrophoretic mobility u measuring the electrophoretic velocity in a unit electrical field.

We will now adopt the Tiselius Unit in our publications and urge others to do so, not only to avoid the wastefulness of the present notation, but also to commemorate the scientist who initiated the far-reaching development of analytical and preparative electrophoretic methodology.

Yours faithfully,

NICHOLAS CATSIMPOOLAS

Massachusetts Institute of Technology

STELIAN HJERTEN

University of Uppsala

ALEXANDER KOLIN

University of California,
Los Angeles

JERKER PORATH

University of Uppsala,
Sweden

news and views

Self regulation of membrane receptors

from Martin Raff

CELLS communicate with each other largely by means of such chemical signals as hormones, neurotransmitters, and growth factors, most of which bind to specific cell surface receptors. The study of these receptors has been revolutionised in recent years by the development of radiolabelled ligands which bind specifically to particular receptors. One of the rewards of this new technology has been the discovery that the concentration of a ligand can regulate the concentration and/or binding properties of its own receptors on the surface of target cells (see Tata, *Nature*, **257**, 739; 1975). There seems little doubt that this is an important homeostatic regulatory mechanism in cell communication, which, until recently, has been largely overlooked.

The first indication that the binding of a ligand could induce the disappearance of a cell surface macromolecule was provided by Beale (*Int. Rev. Cytol.*, **6**, 1; 1957), who showed that antibodies could specifically remove surface antigens from *Paramecia*, probably by causing them to be shed. Several years later, Boyse and Old and their colleagues observed that antibodies against the Thymus-Leukaemia (TL) antigens caused their disappearance from the surface of thymus and leukaemia cells, both *in vivo* (Boyse *et al.*, *J. natn. Cancer Inst.*, **31**, 987; 1963) and *in vitro* (Old *et al.*, *J. exp. Med.*, **127**, 523; 1968). This phenomenon, referred to as 'antigenic modulation', allowed the leukaemia cells to escape from the host's immune response. A similar phenomenon was later described for H-2 antigens and immunoglobulin (Ig) molecules on mouse lymphocytes (Takahashi, *Transplant. Proc.*, **3**, 1217; 1971). By the use of fluorescein (Taylor *et al.*, *Nature new Biol.*, **233**, 225; 1971) and ferritin-coupled (de Petris and Raff, *Eur. J. Immun.*, **2**, 523; 1972) anti-Ig antibodies, it was subsequently demonstrated that modulation of surface Ig molecules (which serve as antigen-specific receptors) on B lymphocytes was due to pinocytosis of the antibody-

antigen complexes; the same mechanism probably accounts for TL modulation (Yu and Cohen, *J. Immun.*, **112**, 1296; 1974). Antigenic modulation is dependent on time, temperature, and antibody concentration; it occurs progressively over several hours and is prevented by various metabolic inhibitors, but not inhibitors of protein synthesis. If modulated cells are cultured in the absence of antibody, the surface antigens return in 6-24 h; and this requires new protein synthesis (Loor *et al.*, *Eur. J. Immun.*, **2**, 203; 1972). Monovalent Fab fragments of antibodies modulate TL antigens (Lamm *et al.*, *J. Immun.*, **101**, 99; 1968) and induce pinocytosis of surface Ig (de Petris and Raff, *Nature new Biol.*, **241**, 257; 1973), which suggests that modulation does not always require cross linking by the ligand, and this distinguishes it from ligand-induced patching and capping of surface macromolecules, for which cross linking is obligatory (de Petris and Raff, *Nature new Biol.*, *op. cit.*).

Although antigenic modulation has provided insight into some properties of cell membranes, its biological significance is still unclear. Recently, remarkably similar phenomena have been observed when some hormones bind to their membrane receptors; the functional implications here are much clearer, the molecular mechanisms less so. Roth and his colleagues have shown both for mice and for men that when serum insulin levels are chronically elevated (for example, in genetic and induced obesity) there is a striking decrease in specific ¹²⁵I-insulin-binding sites on target cells (Kahn *et al.*, *J. biol. Chem.*, **278**, 244; 1973; Soll *et al.*, *J. clin. Invest.*, **56**, 769; 1975). The number of binding sites increases towards normal again when serum insulin is lowered by prolonged restriction of food intake. Conversely, an increase in insulin receptors has been reported in insulin-deficient diabetic hamsters (Hepp *et al.*, *Nature*, **258**, 13; 1975). The loss of specific insulin-binding sites in hyperinsulinemic animals results from a decreased concentration

of surface insulin receptors, rather than from the blocking of receptors by endogenous insulin, the insulin-binding properties of the remaining receptors being entirely normal. Moreover, the *in vitro* exposure of cultured human lymphocytes (line IM-9) to insulin for 5-16 h decreases the concentration of insulin receptors by more than 50% (Gavin *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 84; 1974). This decrease is dependent on time, temperature, and insulin concentration. If insulin is removed from the culture medium, receptors reappear within 12-24 h and the reappearance is blocked by inhibitors of protein synthesis (Lesniak *et al.*, *Clin. Res.*, **22**, 343A; 1975). Recently, Lesniak and Roth (*J. biol. Chem.*, *in the press*) have demonstrated that a similar 'down regulation' of surface receptors for growth hormone (GH) occurs when IM-9 lymphocytes are exposed to human GH *in vitro*, and that insulin and GH receptors can be modulated independently on these cells. Inhibition of protein synthesis with cycloheximide produces a small but progressive loss of GH receptors which adds to the receptor loss induced by GH, suggesting that while the cycloheximide inhibits receptor synthesis, GH accelerates receptor loss. Although it has been suggested that insulin-induced receptor loss may be due to weak intrinsic proteolytic activity of insulin (Huang and Cuatrecasas, *J. biol. Chem.*, **250**, 8251; 1975), the mechanism(s) of insulin and GH-induced receptor loss remains to be established.

Another example in which a hormone induces the loss of its own surface receptors has been described by Hinkle and Tashjian (*Biochemistry*, **14**, 3845; 1975). They have shown that GH₃ cells (a cloned line of a rat pituitary tumour) progressively lose surface receptors for thyrotropin-releasing hormone (TRH) when they are exposed to this hypothalamic tripeptide *in vitro*. These cells, which respond to TRH by increasing prolactin secretion and synthesis, show as much as a 75% decrease in ³H-TRH

binding sites after a 48-h exposure to TRH. Here again, the remaining receptors have the same affinity for TRH as controls, and receptor concentration returns to normal by 96 h after TRH removal. Loss of receptors induced by TRH, like that induced by insulin but unlike that induced by GH, is markedly inhibited by cycloheximide. In this case also, the mechanism of receptor loss is unknown and here it seems unlikely to be due to intrinsic proteolytic activity of the tripeptide hormone.

Recently, the demonstration of receptor regulation by ligands has been extended to a neurotransmitter. It has been known for some time that high concentrations of β -adrenergic catecholamines can induce functional desensitisation of target tissues *in vivo* and *in vitro*, but the detection of changes in the β -adrenergic receptors themselves was delayed by difficulties in preparing specific radiolabelled ligands. The problem of non-specific binding has now been overcome by the use of β -adrenergic antagonists (such as alprenolol) instead of agonists. Using $(-)^3\text{H}$ -alprenolol as a specific ligand, Lefkowitz and his colleagues have shown that prolonged exposure of frog erythrocytes to β -adrenergic catecholamines *in vivo* (Mukherjee *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1945; 1975) or *in vitro* (Mickey *et al.*, *J. biol. Chem.*, **250**, 5727; 1975) leads to progressive and selective decrease (50–70%) in the number of specific alprenolol binding sites without any change in the affinity of remaining sites. Interestingly, whereas β -adrenergic agonists (isoproterenol > adrenaline > noradrenaline) readily modulate their receptors, the potent β -adrenergic antagonist, propranolol, does not; in fact, it prevents the modulation of receptors by agonists. Thus, it seems that receptor occupancy is not sufficient for modulation. The regulation of β -adrenergic receptors differs from that of insulin and growth hormone receptors in that treatment with cycloheximide (*in vivo*, in this case), does not prevent recovery after down regulation (Lefkowitz *et al.*, *Rec. Progr. Hormone Res.*, in the press), suggesting that β -adrenergic receptors may be reversibly inactivated rather than lost. Axelrod and his colleagues have reported similar observations on the *in vivo* regulation of β -adrenergic receptors of rat pineal cells (Kebabian *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3735; 1975). When these receptors are stimulated physiologically (by keeping the animals in the dark to increase the activity in sympathetic nerves supplying the pineal gland) or pharmacologically (by injecting *l*-isoproterenol), there is a rapid fall in specific $(-)^3\text{H}$ -alprenolol binding sites

which is maximal (70% reduction) by 2 h and which begins to recover by 4 h. Exposing animals to prolonged light (which decreases sympathetic activity) increases the concentration of these receptors. In addition, they have shown that the concentration of specific alprenolol binding sites in the rat pineal normally varies with a circadian periodicity that is inversely related to the cycle of neurotransmitter release (Romero *et al.*, *Nature*, **258**, 435; 1975). Thus, there is little doubt that self regulation of neurotransmitter receptors can occur physiologically.

Teleologically, there are obvious advantages in self regulation of receptors. When cells have 'spare receptors' (that is when the maximum response occurs when only a fraction of the receptors is occupied), as is the case in most of the ligand-receptor systems discussed here, it provides an ingenious way for the cell to regulate its sensitivity to a ligand: lowering the concentration of receptor displaces the dose response to the right while still allowing the cell to make a maximal response if exposed to very high concentrations of ligand. If the cell did not have spare receptors, lowering receptor concentration would lower the maximal potential response of the cell. Just as not all surface antigens are modulated by antibody, however, so not all surface receptors are modulated by their ligand. Nor is this type of modulation the only self-regulatory receptor mechanism: a reduction in receptor affinity for its

ligand with increasing saturation of binding sites (so-called 'negative cooperativity') has been described for a number of hormones (De Meyts *et al.*, *Biochem. biophys. Res. Commun.*, **55**, 154; 1973). Moreover, ligand binding is only the first step in the chain of reactions that constitute the cell's response to receptor activation, and it seems likely that regulation in response to varying concentrations of ligand can occur at many of the points in the activation sequence other than at the level of the receptor itself. For example, opiate receptor concentration is not decreased by chronic exposure to high opiate levels (Pert *et al.*, *Science*, **182**, 1359; 1973; Klee and Streaty, *Nature*, **248**, 61; 1974); such 'addicted' cells apparently adapt by increasing the concentration and/or activity of adenylate cyclase to compensate for the rapid and reversible inhibition of adenylate cyclase induced by the initial binding of opiate (Sharma *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3092; 1975). Thus, while some cases of 'desensitisation' (= 'tachyphylaxis' = 'tolerance') induced by chronic exposure to high concentrations of hormones, neurotransmitters and drugs, seem to be due to receptor modulation, others are not. In the examples discussed here, where receptor inactivation or loss is involved, the molecular mechanisms remain to be determined. With the technology now available this should not take too long. □

Invisible astronomy

from David W. Hughes

"WHAT the eye doesn't see the heart doesn't grieve over" is a saying becoming ever less applicable to astronomers. M. E. Bailey, of the University of Sussex (page 290 of this issue of *Nature*) is not of the ranks of radio, infrared, ultraviolet, X-ray and γ -ray astronomers who sightlessly peruse the heavens, but of the smaller band of astronomers who predict the parameters of invisible objects by observing their effect on other stellar and planetary bodies.

One of the first successes in this field was the detection of the white dwarf companion of Sirius A by Bessel in 1844. The wavy paths of astrometric binaries on the celestial sphere has subsequently led to the discovery of planetary companions orbiting about a quarter of the nearby stars to the Sun. Bailey brings us closer to home, into our own Solar System. A great deal is known about the very small and very large objects in interplanetary space.

We observe the small meteorites (diameters normally < 1 m) by the atmospheric trails and surface impact craters they produce when they encounter the Earth. The much larger asteroids, satellites and planets, at the other extreme of the size range (diameters > 1 km) are observable through telescopes. The invisible objects in the Solar System either have sizes between these two limits (10 m < diameter < 1 km) and heliocentric distances less than 5 astronomical units, or are bigger and further away. Both are extremely difficult to detect with telescopes. But when it is considered that Pluto, the faintest planet in the Solar System, has a magnitude +14 and the 200-inch telescope at Mount Palomar, California can detect objects with magnitudes +24 (that is 10,000 times fainter) other planets must have been photographed already, their very small heliocentric velocity as yet rendering them indistinguishable from stars. The detection

of new planets in the Solar System will either be fortuitous or will have to wait until the gravitational effect they have on Pluto and Neptune produces sufficiently large perturbations to enable reasonably accurate predictions of their position to be made.

Cometary nuclei which have a predicted mass range of 10^{18-23} g and a range of diameters from 1 to 100 km have not yet been directly observed. The gaseous coma which surrounds them when they are close to the Sun unfortunately shields the nucleus from observations. Due to the effects of diffraction and contrast the larger comets cannot even be seen when transiting the Sun.

Bailey now suggests a novel way of detecting interplanetary boulders and distant comets. The brightness of stars in the ecliptic plane is continuously monitored to see if the reduction of brightness produced when the star is eclipsed by a nearby interplanetary boulder or cometary nucleus can be detected. This eclipse would last for about the time it takes the object to move its own distance along its orbit; anything from 0.1 s upwards depending on its size and distance from the Earth. A conservative upper limit to the spatial density of the comet cloud, suggests that there are 10^{10} comets in a belt at about 50 AU from the Sun. So using a 1-m telescope, capable of detecting 16th magnitude stars, one of the 1,000 stars visible in the field of view should suffer a short eclipse every 11 h. The problem of picking out this eclipse is an unenviable one.

Another difficulty, inherent in the proposed technique is that a boulder at 1 AU would be indistinguishable from a cometary nucleus at 100 AU. Harwit (*The Zodiacal Light and the Interplanetary medium*, NASA SP 150, 307, 1967) predicts that boulders with sizes between 10 m to 0.5 km have a spatial density of about 2×10^{-18} g cm⁻³. About 10^{24} g of these boulders are thus distributed through a cloud ~ 50 AU in radius and 2 AU thick centred on the Sun. When these get in the way of distant starlight, eclipses will also occur, adding to the number calculated by Bailey. Interestingly Sekanina (in *Asteroids, Comets and Meteoric Matter*, IAU Colloquium No. 22) proposed that the outbursts in brightness observed from comet P/Schwassmann-Wachmann I were produced by boulder impact. This comet has an orbit which always lies between that of Jupiter and Saturn. Evidence for these boulders could come from observations of their collision with other comets, Trojan asteroids or the minor satellite of Jupiter. This would require a similar experiment to that proposed by Bailey although in this case one would be looking for an increase in brightness,

not a decrease. Sekanina points out however that a 10^8 g boulder with a velocity of 60 km s⁻¹ burning out in the Earth's atmosphere would produce a fireball of magnitude +12 when observed from a place 4 AU away so again the effect is marginal. □

Deeper insights into tRNA

from Stephen Neidle

THE transfer RNAs are molecules of central importance in protein synthesis, forming the essential link between the genetic code in the nucleic acids and developing protein molecules. Thus, the determination of the three-dimensional structure of the various tRNA species by means of X-ray crystallography, has received much attention over the past few years. In spite of considerable effort, however, only one has been both successfully crystallised and analysed—the tRNA coding for phenylalanine which has been isolated from yeast. Structural results emerged almost simultaneously from two laboratories—the MRC Laboratory of Molecular Biology, and the Massachusetts Institute of Technology (see *Nature*, **250**, 699; 1974). The Cambridge (UK) group have been investigating a monoclinic form of this tRNA, whereas the Cambridge (USA) workers have studied an orthorhombic one. These analyses have revealed the molecule as adopting very similar overall shapes in both crystallographic environments; indeed it seems likely that even the finest details of intramolecular conformation and inter-base hydrogen bonding, are very comparable. This large measure of agreement thus implies that the electron-density maps obtained have been essentially correctly interpreted by both groups.

They have now reported further analyses of these two structures, which reveal yet more detail. Indeed, so confident are they of their nucleotide-residue assignments that preliminary atomic co-ordinates have been published (Ladner *et al.*, *Nucleic Acids Res.*, **2**, 1629; 1975, and Quigley *et al.*, *Nucleic Acids Res.*, **2**, 2329; 1975). It is considered that the structures are now basically defined although they have not as yet been analysed to the limit of resolution of the diffraction data (about 2 Å), and the full armoury of structure-refinement techniques remains to be applied. The MRC group have also given an account of the 2.5 Å resolution monoclinic tRNA structure (Ladner *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4414; 1975) and the MIT workers have presented further details obtained from their 3 Å electron-

density maps (Quigley *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4866; 1975). It is apparent that the two structures are virtually identical except probably at the 3'-acceptor end of the molecule; this is not surprising, as the sequence here seems to be relatively unconstrained and is able to alter its position in going from one crystallographic environment to another.

Both sets of maps are of sufficient quality for purine and pyrimidine bases to be distinguished from each other; this has also meant that the planes in which the bases lie can be more precisely defined than hitherto. Much of the intricate system of base-pairing has been confirmed, little of which is of conventional Watson-Crick type. It has also become apparent that the hydroxyl groups of many of the riboses play a very important part in helping to augment these tertiary interactions by acting as hydrogen-bond acceptors. Perhaps unexpectedly, it seems that some riboses (possibly as many as ten), adopt a C2'-endo conformation instead of the normal C3'-endo one; details of this will, however, have to await the results of the high-resolution refinements already in progress.

Several new interactions have been revealed, particularly in the neighbourhoods of the highly intricate joins and junctions between loops and stems. For example, guanine-45 seems in both models to be hydrogen-bonded to guanine-10 at the anti-codon arm-D-stem junction. Guanine-10 is itself involved in a Watson-Crick base pair with cytosine-25, thus forming a triplet of bases. Earlier interpretations of the interactions in these junctional regions had several disputed points, but these have now been largely resolved.

In retrospect, the differences and indeed difficulties in map interpretation, were not unexpected bearing in mind the great complexity of the structures, and the fact that these were the first polynucleotide structures to be examined by single crystal analysis. Among the many other points of interest which have emerged from these latest interpretations, is a neat description by the MRC group of a guanine-uracil base pair, which to date has only been observed in this yeast tRNA^{Phe}. Such a base pair was predicted 10 years ago by Crick in his 'wobble' hypothesis, and the glycosidic bond conformational change required to accommodate the wobble seems to be as he suggested. Quigley and his colleagues have detected the probable locations of a number of metal ion sites, which are positioned to interact favourably with phosphates of the polynucleotide chain; as they suggest, such bindings may well play a part in stabilising the chain folding.

A question central to any discussion

THE annual cycle of aggressiveness now demonstrated to occur in several species of rodent (Turner and Iverson, *Ecology*, **54**, 967; 1973, Krebs and Myers, *Adv. ecol. Res.*, **8**, 238; 1974) may have an ecological and even evolutionary function of much wider significance than has hitherto been thought. The species *Clethrionomys gapperi* and *Microtus pennsylvanicus* are found in forest and grassland habitats respectively and seldom occur in the same habitat (Grant, *A. Rev. ecol. Syst.*, **3**, 79; 1972). When populations are not expanding rapidly dispersal is minimal. During periods of expansion considerable dispersal occurs resulting in invasion of normally ignored habitats. Iverson and Turner reported such an event occurring when enlarged *Clethrionomys* populations invaded *Microtus* habitats (*Am. Midl. Nat.*, **88**, 440; 1972), but now it has been shown that either species can be the invader.

Working in spruce forest and old field habitats in Manitoba, Turner, Perrin and Iverson have analysed the components of the tidal effect of an invasion of a *Clethrionomys* forest by *Microtus* and shown the part played by the annual cycle of aggressiveness in returning the situation to normal (*Can. J. Zool.*, **53**, 1004;

1975). During the late autumn of 1973 a significant influx of *Microtus* into forest land was observed. Co-existence with resident *Clethrionomys* occurred throughout the winter but came to an abrupt end in May 1974. Intraspecific aggression of both species fell to its lowest during the winter, and with it the level of inter-

Rodent aggression

from our Animal Ecology Correspondent

specific aggression. In paired encounters neither species was dominant over the other. Dominance was assessed by scoring the number of aggressive acts performed per standard encounter (*Nature*, **247**, 254; 1974). At the start of the breeding season both species became reproductively mature and both intra- and interspecific aggression increased four-fold.

Curiously enough, early in the breeding season *Microtus* became markedly dominant, winning nearly all encounters. That they did not displace the resident *Clethrionomys* is thought by Turner and his colleagues to be due to the (unspecified) advan-

tages provided by the home habitat. By early May all the infiltrators had gone leaving the forest once more to the *Clethrionomys*. It seems that co-existence is possible only when inter-specific competition is at a low ebb and that it is linked to the reproductive cycle. If resource partitioning is of selective advantage in the summer, why is it not of equal importance in the winter? Food for these species is never likely to be limiting although it will fluctuate in abundance during the year, so summer food shortage is unlikely to be the wedge driving each species back to its own habitat. The sudden cease in hostilities, coinciding with the start of widespread dispersal in early autumn, means that very many more individuals survive for much longer than would be the case if temporary, out-of-range residence was not possible. This has two main effects; one is to allow emigrés to continue their search for fresh pastures, with all the evolutionary advantages this may confer (Christian, *Science*, **168**, 84; 1970); the other is to maintain a higher biomass of rodents during the winter than would otherwise be possible. This allows the existence of larger stocks of predators and a correspondingly higher secondary productivity.

of the biological significance of these structural findings is whether the conformation observed in the solid state is a representation of that existing in solution. Chen, Giege, Lord and Rich (*Biochemistry*, **14**, 4385; 1975) have attempted to answer this point by examination of the laser Raman spectra of yeast tRNA^{Phe}. This technique has the advantage over others in that spectra can be observed in both solution and crystal; furthermore, conformational changes can often be readily detected. The observed spectra are almost identical in the two environments. Chen *et al.* also examined the spectra from both the orthorhombic crystals and a hexagonal modification, which again had essentially the same pattern of Raman frequencies and intensities. They conclude therefore that the tertiary structure of this tRNA is not only conserved in different crystal forms, but most importantly, in solution.

The next stages in the tRNA saga must be to relate this structure to its multifarious biological functions. Present attempts are necessarily speculative. Ladner *et al.* have rightly drawn attention to the quite pronounced segregation of the invariant and variable bases at several places in the crystal structure, which they suggest may be involved in synthetase recognition in one instance, and in ribosomal binding in another. It is to be hoped that the

structural studies on synthetases now in progress will answer some of these questions. Kim has also recently turned his attention to synthetase binding (*Nature*, **256**, 679; 1975) and has suggested that symmetry recognition might be important. He has observed that the tRNA crystal structure has approximate two-fold symmetry; sequence data on synthetases suggest that the subunits might also be internally organised into two domains related by two-fold symmetry. It was then straightforward to construct hypothetical symmetrical binding schemes for the two sets of domains in the two molecules; at present, these seem not implausible. □

Making galaxies from stars

from M. G. Edmunds

GALAXIES are made of stars. Perhaps the simplest way to model the formation of a galaxy is to imagine a random distribution of stars in space, add a suitable amount of energy, and let the gravitational forces between the stars go to work. Provided not too much energy is put in, the stars will form a more spatially condensed system—a galaxy. Recently J. R. Gott (*Astrophys. J.*, **201**, 296; 1975) has described some

interesting model building of this social behaviour of stars.

Elliptical galaxies are observed to be spheroidal systems of stars which contain very little gas. Despite considerable variation in the flattening between different galaxies, the distribution of light from the galaxies as a function of distance from the centre of each system is remarkably similar. This observational relation, originally discovered by Hubble (Hubble's Other Law!), can be used to infer the radial distribution of the mass density of stars within a galaxy. Previous simple models of the gravitational contraction, or "collapse", of elliptical galaxies have given too rapid a decline of the density of stars with radius. The disagreement has led other workers to build more complicated models which introduced star formation and gas to affect the dynamics of the collapse. Gott has now resolved the discovery with observation, relying on stellar dynamics only. He assumes that small fluctuations occur in the density of a smooth Universe as it expands from the Big Bang. Stars form and the stars in a region of high density collapse together to form a protogalaxy. The new feature of his model is a continuous increase in the mass of such a protogalaxy by the infall of stars from surrounding regions. One other process is invoked: as the Universe expands, a protogalaxy can experience gravitational tidal forces from

other protogalaxies nearby. Gott uses a very simplified model for the tidal interaction, but it nevertheless suggests two important results. The interaction will limit the final size of the galaxies and also impart angular momentum which will determine the flattening of each system. Although other models can account for the observed structure of ellipticals, Gott claims simplicity in that his models fit the data with fewer arbitrary parameters. His method is also attractive because it does not introduce any assumptions about star formation, other than that it occurs before collapse of the protogalaxy. Models which introduced star formation as a variable had to employ very *ad hoc* assumptions since no coherent theory of star formation exists.

The step from a theory of elliptical galaxy formation to a theory of spiral galaxy formation is a dangerous one. The main feature of spiral galaxies is a flat spiral disk of gas and bright young stars. A popular view, restated by Gott, is that spirals form if there is a significant amount of gas remaining in the system during the initial collapse, contrasting with the formation of ellipticals where effectively all the matter is incorporated into stars before collapse. The stars behave as in the collapse of an elliptical system, and a spheroidal distribution forms. But the gas interacts strongly with itself, rapidly dissipating much of its kinetic energy and forming a thin disk within the spheroidal "halo" of stars, rather like the meat inside a hamburger. Subsequent birth of stars from the gas gives the bright observed disk, but these stars have fairly small velocities and stay in the disk since the kinetic energy of the gas out of which they formed has been dissipated during the collapse. The halo of old stars remains.

The idea that massive haloes containing an equal or major part of the mass exist around the disks has become respectable as a result of several studies of the stability of the disks. A massive halo can stop the rapid evolution of a disk of stars into the bar-like structures which occur in numerical simulations of the stellar dynamics of disk systems. Since it is believed that spirals are not just a very transitory stage of galactic evolution, such instability of the disks is undesirable. In Gott's picture of galaxy formation the existence of such stabilising haloes around spirals is quite natural. But an attempt to determine the scale of the halo around our own Galactic disk by Maarten Schmidt (*Astrophys. J.*, **202**, 22: 1975) implies that the mass of our halo is much less than the mass of our disk. Stars in the halo possess on average much higher velocities relative to Earth than do disk stars. Schmidt uses a high velocity criterion to

identify halo members near the Sun. By making fairly reasonable assumptions about the relation between mass and luminosity for these old halo stars he infers that the local density of halo stars is at least an order of magnitude less than the mass required to stabilise the disk. The statistics of such an investigation are necessarily poor, but his result certainly indicates that mechanisms to stabilise the disk, other than the existence of a massive halo, should be sought. The result also implies that for spirals like our own Galaxy, Gott's simple stellar dynamics of spheroidal collapse which seems to work so well for ellipticals, is only a small part of the formation process. It is the material that forms the disk, rather than the halo, which will dominate the gravitational interactions during the collapse, which together with gas interactions will make a theory of spiral galaxy formation considerably more complicated. □

Superfluid ^3He : an impediment removed

from P. V. E. McClintock

WITH the publication in *Physical Review Letters* (**35**, 615; 1975) of a comment by Webb, Sager and Wheatley of the University of California at La Jolla, one of the principal impediments to the identification of superfluid $^3\text{He-B}$ as the state originally proposed by Balian and Werthamer (*Phys. Rev.*, **131**, 1553; 1963) seems to have fallen away.

Since its discovery in 1972, there has been an explosion of theoretical endeavour towards gaining a satisfactory understanding of superfluid ^3He and, considering the inherent difficulties of work in the relevant temperature range below 2 mK, a quite astonishing range of experimental data has also been acquired. As a result it is now known that in a weak magnetic field there are two principal superfluid phases: the A-phase, which is stable only within a limited range of temperatures (T) for pressures (P) between 22 bar and the solidification pressure of 35 bar; and the B-phase, which occupies the greater part of the P - T phase diagram for the liquid. Both phases exhibit complicated magnetic properties, and both are highly anisotropic, being in many respects akin to liquid crystals, with the orientations of their axes of anisotropy being determined by such factors as the applied magnetic field, thermal currents, and the proximity of the walls of the containing vessel.

The present theoretical picture of the superfluid phases, which has been constructed mainly on the basis of their observed magnetic properties,

portrays the liquid as having much in common with the superfluid electron gas in a superconducting metal, in that the superfluidity arises through the formation of pairs of particles. In contrast to the superconducting situation, however, it has been clear that the ^3He atoms form pairs whose relative angular momentum is non-zero. Thus, the two atoms of a pair can be regarded as orbiting around each other somewhat like a diatomic molecule (but the analogy should not be pressed too far: unlike a diatomic molecule, the correlated pair of ^3He atoms spends most of its time separated by distances very much larger than the average interatomic spacing in the liquid). Each pair in the superfluid is in the same quantum state. Thus, to change the state of one pair, for example by setting it in uniform translational motion, would involve simultaneously altering the state of every other pair which clearly is a relatively difficult thing to do, thus providing an insight into the origin of the observed superfluid properties.

Further complications arise because the ^3He atomic nucleus carries a net spin and hence an associated magnetic dipole moment. Much of the theoretical effect has been aimed at discovering exactly how the orbital angular momentum of a pair is correlated with the orientations of its nuclear spins, as well as at finding the answer to the more fundamental question as to the magnitude of the orbital angular momentum itself. It became accepted at quite an early stage that the A-phase was probably in the state first suggested by Anderson and Morel (*Phys. Rev.*, **123**, 1911; 1961) and subsequently developed and justified in more detail by Anderson and Brinkman (*Phys. Rev. Lett.*, **30**, 1108; 1973). In this (ABM) state, the pair orbital angular momentum is unity, and the nuclear spins lie in the plane of the orbit either parallel ($\uparrow\uparrow$) or antiparallel ($\downarrow\downarrow$) to an externally applied magnetic field; there are no pairs with opposed ($\uparrow\downarrow$) nuclear spins. The B-phase was identified, although with less certainty, as a form of the (BW) state originally proposed by Balian and Werthamer in which the pair angular momentum is again unity, but all three possible spin states ($\uparrow\uparrow$, $\downarrow\downarrow$, $\uparrow\downarrow$) are present and the correlation of spins and orbit is somewhat more complicated.

Associated with both the A and B phases are characteristic frequencies f_A and f_B , dependent on T and weakly on P , which arise when the equilibrium configuration of the liquid in a magnetic field is slightly disturbed. These frequencies can be measured directly in longitudinal nuclear magnetic resonance (NMR), can be deduced from the shift of the transverse NMR frequency from its value in normal (non-

superfluid) liquid ^3He , or can be measured from the magnetic ringing which follows a small incremental change in the applied magnetic field. A crucial test of the validity of the ABM and BW state assignments which was suggested by Leggett's calculations (*Phys. Rev. Lett.*, **31**, 352; 1973) involved measuring the ratio f_B/f_A at the A-B transition: according to his equations, the quantity $(f_B/f_A) \times (\chi_B/\chi_A)$ should be precisely equal to $(5/2)^{1/2}$, or about 1.58. Here, χ_A , χ_B are the static magnetic susceptibilities of the two phases.

Subsequent magnetic ringing experiments at La Jolla by Webb, Kleinberg and Wheatley (*Phys. Rev. Lett.*, **33**, 145; 1974) gave a value for f_B/f_A of 1.9 ± 0.1 near the polycritical point (at which the A, B and normal phases are in mutual equilibrium) where $\chi_A = \chi_B$ to an excellent approximation. This unwelcome result received support from work at Helsinki by Ahonen, Alvesalo, Haikala, Krusius and Paalanen who reported (*Phys. Lett.*, **51A**, 279; 1975) that, for similar conditions of P and T , $f_B/f_A = 1.93 \pm 0.05$. These results were disconcerting in that they seemed unambiguously inconsistent with $^3\text{He-B}$ being in the BW state, assuming that one had accepted as correct the identification of $^3\text{He-A}$ with the ABM state.

On the other hand, Osheroff (the original discoverer of the superfluid phases during his PhD research at Cornell University) and the group at Bell Laboratories, working at the solidification pressure in a compressional cooling cell, were persistently reporting NMR results which seemed entirely consistent with the BW identification of B-phase. In particular, Osheroff (*Phys. Rev. Lett.*, **33**, 1009; 1974) found very close agreement with Leggett's $(5/2)^{1/2}$ prediction. Thus, a situation arose where Bell Laboratories data at 35 bar seemed to support the BW state, whereas data taken at lower pressures in two other laboratories appeared to show that this state assignment was incorrect.

The overall situation was particularly disquieting because, as Leggett pointed out (*Rev. mod. Phys.*, **47**, 331; 1975) it occurred "in an area where neither theory nor experiment appears to have much room to manoeuvre". In the event, the theory and the Bell Laboratories group have stood firm and it is the experimenters at La Jolla who are doing the manoeuvring, as may be seen in the comment from Wheatley's group: their new measurements of magnetic ringing, again near the polycritical point but using a different experimental geometry, have yielded results which appear to be in reasonably good agreement with Leggett's $(5/2)^{1/2}$ factor. It is said that a similar

Dynamo attack

from Peter J. Smith

WHEN the principle of the self-exciting dynamo was put forward in the late 1940s to explain the origin of the Earth's magnetic field, the core motions required to maintain the dynamo were attributed to thermal convection generated by radioactive heating. Much later, Malkus (*J. geophys. Res.*, **68**, 2871; 1963) proposed alternatively that the driving force on the core could be the Earth's precession. The idea of precession-induced flow was not new, for the early dynamo theorists had considered and rejected it. What Malkus did was to show that the original reasons for rejecting a precession-driven dynamo were unsound, since which time both convection and precession have been widely accepted as serious contenders for the role of core driver.

Now, however, Rochester *et al.* (*Geophys. J.*, **43**, 661; 1975) report calculations which appear to prove that the power to be derived from precession is at least an order of magnitude too low to stir the core into stable flow. This disagreement with Malkus would be interesting in its own right; but the report is all the more remarkable in that Rochester and his colleagues go on to criticise Malkus severely not only for his result but for the way he obtained it. Specifically, they accuse Malkus of appealing to dubious analogies, of claiming agreement with previous work where no such

agreement exists, of publishing inconsistent equations, of error in mathematical logic, of mathematical oversimplification and of numerical errors, among other things. They further object that in later articles Malkus has not only ignored the few published objections to his 1963 work, but has repeated the errors and retracted nothing.

This is the most severe attack to have appeared in the Earth sciences for many years and raises ghosts from another century. But whether or not one agrees with the form of the criticism, it would be a pity to overlook the serious issue it raises. Many people have come to believe in the feasibility of a precessionary dynamo because they have read Malkus's conclusion but not the arguments on which it is based. The problem is that studies in this field are so esoteric that only a handful of people in the world can understand them; indeed, even Rochester and his colleagues claim not to be competent to deal with all the points involved. As they themselves point out, under such circumstances the rationale for avoiding unpleasant criticism, as described by Ravetz (*Scientific Knowledge and its Social Problems*, OUP, 1971), breaks down and myths develop.

The question now is: is the precessionary dynamo such a myth or not? For the irony is that most Earth scientists will find the detailed arguments of Rochester *et al.* no easier to understand than those of Malkus, and will, as before, have to be content with the conclusion.

manoeuvre has also been carried out in Helsinki. The reason for the earlier discrepant results is not yet entirely clear, but they can perhaps be regarded as a reminder of how much is still not properly understood about the liquid.

The new measurements at La Jolla, which provide a welcome vindication for the work of Osheroff and the Bell Laboratories' group, may be regarded as greatly strengthening the present state identifications of both superfluid phases of liquid ^3He and will, no doubt, have been received with sighs of relief from the many theorists working in the field. □

Regulatory genes and quantum evolution

from A. Hallam

THE abrupt appearance of higher taxa in the fossil record has long been something of an enigma to palaeontologists. Although it is true that temporal sequences of fossils provide some of the

best evidence of evolution, Darwin himself was somewhat embarrassed by the sparsity of transitional forms, which he attempted to explain away by invoking numerous erosional breaks in the sequence of strata bearing the fossils. Since Darwin, many of these 'stratigraphic' gaps have been filled as a result of research throughout the world by numerous people, yet the quantum jumps between phyla and lower taxa by and large remain. A few decades ago Simpson made a valiant attempt to grapple with the problem by putting forward an ecological model of 'quantum evolution' consistent with current genetic theory. This involved small populations of particular species crossing some environmental threshold into a new adaptive zone (certain mammals re-entering the sea for example), whereupon there ensued rapid evolutionary radiation and hence pronounced morphological divergence from the parent stock.

Valuable as Simpson's ideas have proved, major problems and uncertain-

ties have remained about the underlying genetic control, and further attempts must be made to square the facts of palaeontology with the new knowledge and insights gained from molecular biology. Evolutionary genetics has been primarily concerned with structural genes, those genes which are transcribed into RNA, leading to the synthesis of particular proteins and enzymes. It turns out however that there are striking similarities in the basic proteins and enzyme activities throughout the organic world, from primitive prokaryotes to mammals, which vastly outweigh the differences. The enormous differences within the animal and plant kingdoms are much more a matter of organisation and structure. This suggests to Valentine and Campbell (*Am. Sci.*, **63**, 673; 1975) that the evolutionary divergence of major and perhaps even minor taxa has been effected mainly by changing patterns of gene regulation rather than the mere substitution of amino acid sequences in proteins.

The regulatory model proposed for prokaryotes (bacteria and blue-green algae) by Jacob and Monod, involving operator and repressor genes, has been verified in many particulars, but gene regulation in the much more complex eukaryotes (the rest of the organic world) is far less well understood. Valentine and Campbell favour the general regulatory model for higher organisms put forward by Britten and Davidson (*Q. Rev. Biol.*, **46**, 111; 1971). 'Sensor' genes receive a stimulus, probably hormonal, and cause 'integrator' genes to transcribe. The transcription product, activator RNA, is detected by 'receptor' genes, which in turn cause structural genes to transcribe. Whole batteries of receptors, which need not be mutually exclusive, operate under the control of a single integrator, and a hierarchy of sets could be controlled by a series of master sensors. It is evident that mutations affecting regulatory genes, together with chromosomal rearrangements, could create in a geologically short time a wide variety of novel patterns involving large numbers of structural genes.

In broad outline evolution can be envisaged to have proceeded in a number of major steps, each involving a repatterning of genes in new combinations and expansion of the regulatory apparatus, as cell types differentiated and complex body-layer and organ systems progressively developed. Thus for the animal kingdom the sequence is: protozoans; diploblastic metazoans (sponges, jellyfish); triploblastic metazoans (flatworms); primitive burrowing coelomates (many worms); advanced coelomates, often with skeletons. Each successive step involved significant addition of new in-



A hundred years ago

OUR readers no doubt know that we have a younger French sister who appears under the name of *La Nature*. We have just received from Germany a specimen of another of the family, rejoicing in the name of *Die Natur*. This seems, however, to be a new series of an old-established journal, but whether it has always appeared under its present name we cannot make out. It is conducted by Dr. Otto Ule and Dr. Karl Müller, of Halle, is mainly devoted to natural history, and the number sent us contains several interesting articles; among these is one on the African Steppes, by Dr. Ule. from *Nature*, **13**, January 27, 256; 1876

formation to the regulatory apparatus and creation of batteries of structural genes in new combinations. The fossil record indicates that all the steps were accomplished by the end of the Cambrian, some 500 million years ago, when all but two of the living phyla that are well skeletonised had already appeared. This does not mean that most palaeontologists are necessarily only concerned with the equivalent of philosophy's 'footnotes to Plato', because there is still a vast amount to be discovered about the detailed patterns of evolution as revealed by fossil distributions in time and space. The Valentine and Campbell paper is valuable not so much in prediction as in providing a satisfying interpretive framework for further research. □

Rapid quenching: second harvest

from Robert W. Cahn

The Second International Conference on Rapidly Quenched Metals was held at the Massachusetts Institute of Technology on November 17-19, 1975. It was organised by Professor N. J. Grant, of MIT, and Professor B. C. Giessen, of North-eastern University.

WHEN a canny farmer ploughs a new field, the first harvest will as likely as not be bounteous; but the second harvest defines the long-term value of the land. The Conference was dedicated to Pol Duwez, the American metallurgist who first opened up the field of

rapidly quenched metals 15 years ago; a rich first harvest was reaped in 1970 during the First Conference, and now the second has proved that he made a sound investment.

The Conference was immediately followed by a Workshop (held at North-eastern University, Boston) devoted to the applications of amorphous alloys. These materials—also known as metallic glasses—are merely one sub-category of rapidly quenched metals, but the fact that two days were entirely devoted to them reflects the organisers' judgment, vindicated by the Conference and Workshop, that the corn now grows tallest in this corner of the field.

The first Conference, in 1970, was largely concerned with extended solid solubility and with anomalous inter-metallic phases resulting from splat-quenching of alloy melts. Fewer papers this year dealt with these concerns than one would have foretold then. Some of these were concerned with steels: groups at Cambridge (UK), Tohoku University (Japan), and the University of Pennsylvania (USA) examined the behaviour of splat-quenched steels, both in their initial state and after subsequent transformation: crystallography, morphology and kinetics were all investigated. The Cambridge group concentrated on familiar steels such as Fe-Mo-C steels, and observed solubility extension in ferrite, retained austenite and a number of unfamiliar carbides: the steels were at all stages crystalline. The others examined Fe-P-C, one of the transition metal/metalloid combinations which become amorphous on quenching, and investigated the details of subsequent crystallisation. This is an area where much more research is now needed, with good prospects of industrial applications.

Aluminium alloys continue to be the favourites among those who prefer to investigate crystalline forms of metastability. Some of these have reached the verge of industrial use. A group at the Battelle Institute in Frankfurt (Germany) have scaled up a technique for splat-quenching and compaction of Al-Fe alloys (for some years now the preferred alloys for practical application), further alloyed with Mg, Mn and Cr. The product is not only very strong, even at 300 °C, but—like a number of other chromium-bearing splat-quenched alloys—extremely resistant to corrosion: there was no detectable attack by superheated seawater at 150 °C. Some applications are immediately apparent. A group from the University of Sussex (UK) showed how a plasma-spraying gun, with improvements to help keep the deposit cool, can be used to spray highly super-saturated and thus extremely strong Al-Cu alloy sheets.

An impressive paper from Battelle

Pacific Northwest (USA) showed how a large sputter-deposition apparatus can be used to make sizeable artefacts of metastable materials, including alloy glasses. This seems to be the only available way of making such objects other than in thin-sheet form.

Surprisingly little attention was paid to the regularities governing solubility extension and metastable intermetallic phases: one paper from Northeastern University identified some relationships between stable and metastable ordered intermediate phases, and a major paper from Carnegie-Mellon University (USA) pinpointed the influence of 'electronic factors' à la Hume-Rothery on the extension of solid solubility in various alloy systems. In summary, unfavourable size factors can be overcome by rapid freezing but unfavourable electronic factors cannot.

Over the past five years there have been notable developments in quenching techniques. A good deal of interest was manifested in the technique of plasma-spraying. The high point, however, was a paper from the Battelle Laboratories in Ohio (USA) describing a patented new method for making rapidly quenched ribbons. A rapidly rotating contoured wheel dips into a metallic melt, either subjacent in a crucible or pendant over the wheel from a rod of solid metal: the wheel 'spins' out a ribbon which lifts clear after it has frozen. The ribbon can either be spooled or else the wheel can be provided with a series of circumferential nicks; when that is done, a shower of short filament lengths is generated, ready to substitute for chopped fibre in (for instance) reinforced concrete. This technique has already been licensed and is soon to go into industrial production. Though the method has required a good deal of fine tuning it has the essential simplicity of all classic production techniques and will be a significant rival to the roller-casting technique, already in commercial use by Allied Chemical Corporation to make their 'Metglas' amorphous alloys. That Corporation's scientists were much in evidence during the five days, and a number of new compositions were announced, in particular Metglas 2605 ($\text{Fe}_{80}\text{B}_{20}$), notable for high magnetic permeability and high strength, and a range of Be-Ti-Zr glasses such as the high-strength Metglas 0422, $\text{Be}_{40}\text{Ti}_{50}\text{Zr}_{10}$.

Much attention was devoted to the structure of amorphous alloys (a piquant concept, since 'amorphous' derives from the Green root for 'formless'). Several laboratories, notably the Tohoku group, the Technical University of Berlin and the University of Paris-Sud, have firmly established by X-ray and neutron diffraction and other methods that alloy glasses have

very pronounced compositional short-range order, as had been suspected. The interest evident five years ago in computer-modelling of alloy glasses based on 'dense random packing' (with a view to comparing computed and experimentally derived pair distribution functions) has been fully maintained, while the rather pointless semantic controversy between the 'true amorphous' and 'microcrystalline' models shows signs of attenuating. In this field of research, scientists from the IBM Watson Research Centre at Yorktown Heights (USA) and the long established group at Harvard are making major contributions.

More than half of the Conference/Workshop was devoted to the mechanical and magnetic properties of alloy glasses. The combination of very high strength with appreciable ductility of these materials has been well documented, and no surprises emerged. Interest is beginning to focus on the influence of annealing, below or above the crystallisation temperature, on mechanical behaviour. One intriguing observation was that annealing can embrittle phosphorus-bearing, deformed, alloy glasses, and controversial evidence suggests that phosphorus diffuses to shear bands and promotes fracture.

The new Be-Ti-Zr ribbons will be of particular interest. One novelty was the observation that fracture toughness is a sensitive function of ribbon thickness, and is an optimum for a thickness less than the maximum which can be fabricated. This is not as yet understood. Although the makers of 'Metglas' ribbons have for some time now emphasised the role of their product as a reinforcing fibre, this Conference heard the very first public account of a systematic attempt to put this claim into effect. A group at Pratt & Whitney Aircraft experimented with epoxy resins reinforced with one of the commercial ribbons, up to 40% volume fraction; though the resin-ribbon bond was weak, good reinforcement was attained both longitudinally and transversely. It is astonishing that this should be the first published investigation of its kind, and that no accounts of filament-winding have yet appeared.

The topic which now excites the greatest interest, both scientifically and industrially is the ferromagnetic behaviour of various amorphous alloys based on iron and nickel, and to a lesser extent cobalt. A great many contributions centred upon this, especially an impressive cluster from Bell Telephone Laboratories (Murray Hill, USA) and another from the General Electric R and D Centre at Schenectady (USA). An academic group at the University of Pennsylvania and the

extremely energetic Japanese group at Tohoku University are also active in this field. Increasingly, the various groups are manufacturing their own glassy materials. (If two individuals may be picked out for sheer collaborative productivity in research on amorphous alloys, honours should go to H. S. Chen of Bell Telephone and T. Masumoto of Tohoku).

The major group of alloy glasses—including now the new commercial $\text{Fe}_{80}\text{B}_{20}$ —has high permeability, low coercivity and reasonably good performance at high frequencies. Materials with zero magnetostriction and consequently extremely low anisotropy energies and high permeabilities, are now routine. A wide range of magnetic properties is in fact attainable in one and the same alloy, merely by changing the details of the magnetic annealing treatment.

Magnetic domains are detected in spite of ultra-low anisotropies and the ribbons are sensitive to handling, though less so than materials such as crystalline permalloys (as one exasperated scientist remarked, one dare not look at permalloy for fear of spoiling its permeability!) Roller-cast ribbons have periodic variations of magnetic behaviour, related to periodic variations of internal stress introduced during production. The intimate symbiosis between minutiae of manufacture and magnetic behaviour, long familiar in the world of magnetic materials, is again very much in evidence. A newly discovered family of glassy alloys based on Fe/Th compositions and made by massive sputter-deposition, prove to be strong permanent magnets. Here high anisotropy appears to stem from local variations in composition and structural order.

As the GE group in particular indicated, there are in principle very extensive magnetic applications for alloy glasses in industry, especially the high-permeability kind. Filter chokes, transducers, loading coils, fluxmeters, magnetic amplifiers, L-F inverters and indeed transformers are all candidates. Much of the ground work in establishing suitable compositions and forms of magnetic annealing has been done. There is just one remaining obstacle, but it is a major one! Up to the present, alloy glass ribbons can only be made a few millimetres wide. For most applications, wide sheet is essential. The commercial people present were extremely tight-lipped about this crucial problem, which seems to be very obstinate. A very large prize, indeed, awaits the processing specialist who is first able to resolve this impasse, either by inventing a new form of continuous quench-casting or by some totally different approach. □

review article

Differential function of major histocompatibility complex antigens in T-lymphocyte activation

Fritz H. Bach, Marilyn L. Bach & Paul M. Sondel

The antigenic systems of the major histocompatibility complex can be subdivided into those which are serologically detectable and those which are detected in tests with mixed lymphocytes. The two systems have different roles in the activation of separate populations of T lymphocytes.

FEW investigators attracted to the study of transplantation antigens controlled by the major histocompatibility complex (MHC) foresaw the multitude of fascinating biological phenomena related to genes of this region. MHC loci are involved not only in the control of strong transplantation antigens, but also in control of immune response, disease susceptibility, immune cell interactions, level of serum complement components and perhaps in developmental abnormalities; it seems that many of the genes determine cell surface structures. This review will deal largely with antigens controlled by the MHC, their genetic control and function in immunological reactions.

MHC associated antigens were originally detected by transplantation *in vivo*; the recognition of serologically defined antigens controlled by genes of that same genetic complex led to the question whether these antigens were themselves the strong histocompatibility (transplantation) antigens or genetic markers for them. Additional serological studies as well as the use of two *in vitro* models of cell-mediated immunity have provided evidence for the existence of cell surface antigens determined by MHC genes other than those controlling the initially described serologically defined antigens. The accumulated data suggest that different antigens of the MHC are separable on the basis not only of the recombinational events separating the genes coding for them, but also of different functional roles in terms of lymphocyte subpopulations that respond to them.

Detection of MHC antigens

The two *in vitro* cellular models that have most extensively been used for the analysis of MHC associated antigens are the mixed leukocyte culture (MLC)^{1,2} and cell-mediated lympholysis (CML)³⁻⁶ assays (Fig. 1). In MLC tests, lymphocytes from one individual (the responder) are cultured for 4-7 d with stimulating lymphocytes from a second individual. To prevent their proliferation, stimulating cells are treated with mitomycin-C (ref. 7) or X rays⁸ before they are added to responding cells. When the stimulating cells are from individuals whose MHC is different from that of the responder the untreated responder lymphocytes proliferate; this is detected by incorporation

of tritiated thymidine (³H-TdR) into the proliferating cells in the MLC assay. For CML assays, cytotoxic T lymphocytes (CTLs) are "generated" in mixed leukocyte cultures⁹⁻¹¹. After incubation for several days, the responding cells are assayed as effector lymphocytes for their cytolytic activity as measured by their ability to lyse radioactive sodium chromate (⁵¹Cr) labelled target cells; if the target cells carry the same (or cross reactive) antigens as the stimulating (sensitising) cells in MLC, then the target cells are lysed¹². The amount of ⁵¹Cr released into the medium is a quantitative assay for lysis and is expressed as CML. Since the terminology in this field is complex and can be difficult to follow, terms and abbreviations used in this article are listed in Table 1.

On the basis of serological findings and the use of MLC and CML tests, it is possible to divide MHC determined cell surface antigens into at least three categories. First, the classical serologically defined antigens that are present on the surface of essentially all cells. We shall refer to these as the SD antigens. Second, some antigens which can be detected serologically are present only on restricted cell types, among them B lymphocytes, macrophages, epidermal cells and sperm¹³. These antigens are referred to as Ia (Ir-associated) in mouse and by other designations in man. Although it has not been conclusively established that these mouse and human antigenic systems are homologous we shall make that assumption and, since the human terminology has not been unified, refer to both as Ia. Third, there are the MHC antigens that, if different in two individuals, lead to an MLC proliferative response^{14,15}. We shall refer to these as LD antigens^{15,16}. Whereas genes determining the MHC SD antigens are genetically separable from those determining the Ia and LD antigens in both mouse and man, there may be some degree of identity between Ia and LD determinants.

It must be stressed that the designations SD, Ia and LD are simply terms we use to distinguish between MHC determinants that may have different biological roles. Since the terminology for these antigens and the loci coding for them are different in the several experimental species used for various studies, the terms allow simplified reference to and discussion of pertinent phenomenology related to what are presumably homologous systems in several species. In addition, in a single species several genetically separable loci coding for antigens all apparently subserving a single function can be conveniently referred to with a single

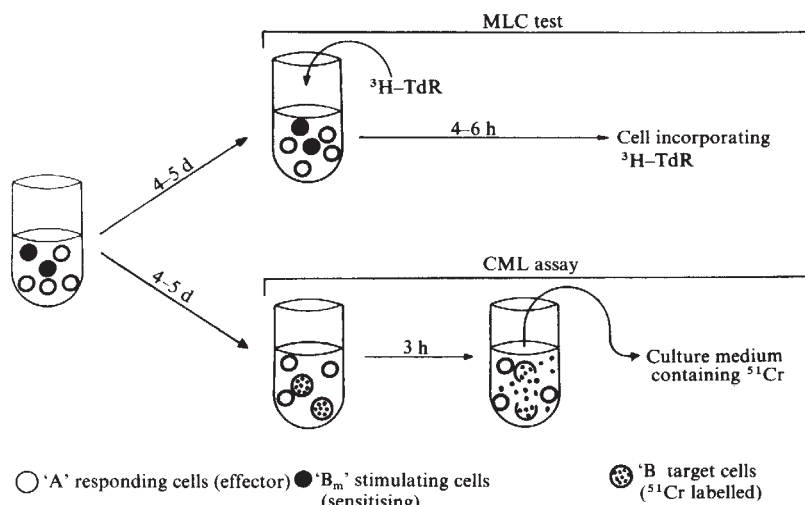


Fig. 1 Schematic representation of the MLC and CML tests. Cells differing for the major histocompatibility complex are mixed *in vitro*: for the MLC assay, proliferative activity is assayed after 4–5 d by studying incorporation of radioactive thymidine; for the CML assay, the initial responding cells are tested for their cytotoxic capacity against radioactively labelled target cells, the amount of radioactive label released from the target cells by the effector cells is expressed as % CML.

term. The terms should in no way imply that a function associated with LD cannot also be associated with SD; for example, that SD antigens cannot induce lymphocyte proliferation. On the premise that the broad biological principles discussed in this article are the same in mouse and man, we shall draw freely on data obtained in both species.

Genetics of the MHC in mouse and man

The MHC in mouse, called *H-2*, has been divided into five regions^{13,17}, K, I, S, G and D on the basis of marker loci for each region (Fig. 2). The K and D regions have as their marker loci *H-2K* and *H-2D*, that control the *H-2* SD antigens. The I region can be subdivided on the basis of different Ia determinants associated with each of three sub-regions: I-A, I-B and I-C^{13,14,17,18}. Included in I-A are loci determining certain Ia antigens, an immune response (*Ir*) locus controlling the ability of an animal to respond immunologically to a variety of antigens, and the strongest *H-2* LD locus, that is differences for this locus lead to strong proliferative responses in MLC. Within this subregion the genes determining Ia antigens, LD determinants, and controlling immune response have not been genetically

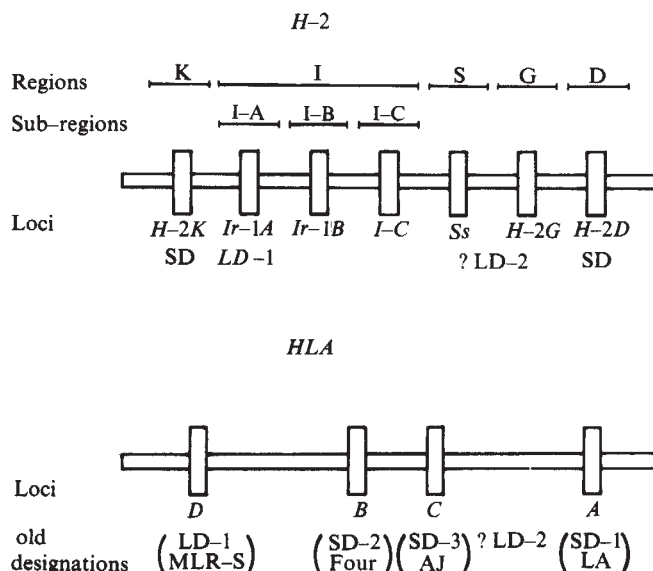
separated. Partial identity of *Ir* and Ia genes is suggested by the existence of a soluble factor that bears Ia antigen determinants and that is involved in regulation of the immune response^{19,20}.

The human MHC, termed HLA (Fig. 2) has three different SD loci, *HLA-A*, *HLA-B* and *HLA-C* and one strong LD locus, *HLA-D*; a weak LD locus may exist between *HLA-A* and *HLA-C*. Van Leeuwen *et al.*²¹ and more recently Winchester *et al.*²² have demonstrated the existence of cell surface antigens determined by genes of the human MHC that at least in part have a similar tissue distribution to the mouse Ia antigens.

For convenience in further discussion, the word "locus" will be used to describe the genetic control of all antigens or determinants where it has not been shown that more than one cistron is involved. Two strains (individuals) designated "LD different" differ for the strong LD locus and are identical for the SD loci; those designated "SD different" differ for either the K and/or D regions in mouse or the SD loci in man but are identical for the strong LD

Fig. 2 Diagrammatic representation of the major histocompatibility complex in mouse and man. In mouse, the complex is divided into five regions with the I region further divided into three sub-regions. Marker loci for each of the sub-regions are shown. The strongest LD locus is in the I-A sub-region; a weaker LD locus may be present between *S_s* and *H-2D*. The *H-2D* and *H-2K* loci are the SD loci.

Table 1 Abbreviations and terms	
MHC	Major histocompatibility complex, <i>H-2</i> in mouse and <i>HLA</i> in man.
<i>Ir</i>	Immune response loci alleles which control the ability of an animal to respond to a variety of antigens.
Ia	The antigens controlled by the <i>H-2</i> I region in mouse that are primarily expressed on B lymphocytes, macrophages, epidermal cells and sperm. An apparently homologous antigenic system has been described in man.
MLC	Mixed leukocyte culture test; usually assayed by study of thymidine incorporation into stimulated lymphocytes.
LD	The MHC loci and antigens primarily responsible for inducing thymidine incorporation (cell proliferation) in MLC. The stronger of them are controlled by <i>HLA-D</i> in man and <i>LD-1</i> in mouse.
PHCs	Proliferating helper cells; the T lymphocyte subpopulation that is primarily responsive to MHC LD antigens. These T cells do not adhere well to allogeneic monolayers and carry the Ly-1 differentiation antigens.
CML	Cell-mediated lympholysis assay, assayed by studying release of ⁵¹ Cr from labelled target cells.
SD	The MHC loci and antigens (or genetic markers for the antigens) that serve as the primary targets for the cytotoxic T lymphocytes. These are the classical serologically defined antigens of <i>H-2K</i> and <i>H-2D</i> in mouse and <i>HLA-A</i> , <i>-B</i> and <i>-C</i> in man.
CTLs	Cytotoxic T lymphocytes active in the CML assay. These T cells adhere to allogeneic monolayers and carry the Ly-2 and Ly-3 differentiation antigens.



locus. This focuses on only the MHC LD and SD loci; other MHC loci may well be involved in the reactions discussed.

Genetic control of MLC

In man, the *HLA-D* (*LD-I*) locus has the predominant role in inducing proliferation in MLC; differences for the SD loci do not, by themselves, lead to strong MLC activation (reviewed in refs 23 and 24). Cells of siblings that are *HLA-D* identical but differ for SD loci either do not stimulate each other at all or do only minimally^{25,26}; this low stimulation may be due to SD antigenic differences themselves and/or to a weak LD locus between *HLA-A* and *HLA-C*. It is not established whether *HLA* SD antigens provide a significant additional proliferative stimulus in MLC in the presence of *HLA-D* disparity.

In mouse, a strong LD locus in the *H-2* I region is primarily responsible for proliferative events in MLC; differences for the *H-2* SD regions (K and/or D) given I region identity are also significantly stimulatory although to a lesser extent. These latter proliferative responses, as in man, may be caused by the SD antigens or by LD-type stimuli within the K and D regions (for example, there is evidence for an LD locus between S and D²⁷). In mouse, apparently unlike man, there is a locus (*Ms*) alleles of which segregate independently of *H-2*, and differences at which lead to an MLC proliferative response²⁸. Even in xenogeneic combinations, such as man-mouse, the same *H-2* regions that lead to an allogeneic MLC reaction provide the proliferative stimulus (K. F. Lindahl and F.H.B., in preparation).

Genetic control of CML

Despite the finding that the MHC LD antigens are primarily responsible for activating the proliferative response in MLC, and that cytotoxic T lymphocytes (CTLs) active in CML are generated in MLC, the target antigens primarily recognised by CTLs are not LD. Rather the specific lytic effect of CTLs is directed at either the MHC SD antigens themselves or the phenotypic product of genes very closely linked to those determining the SD antigens²⁹⁻³³. This is, once again, true even in xenogeneic combinations³⁴. We shall, for simplicity, refer to them as the SD antigens.

These findings are most elegantly demonstrated in "three-cell" experiments^{35,36}, in which cells of one strain (individual) are simultaneously stimulated by mitomycin-C treated cells of two other strains, one differing from the responding cells by LD and the second differing by SD antigens (Table 2). In addition these data give evidence for "LD-SD collaboration". Cells of two LD different strains (AQR-B10.T(6R)) stimulate each other significantly in MLC but the LD stimulus by itself does not generate cytotoxic cells directed at the SD target. Two SD region different strains (AQR-B10.A) lead to a relatively weaker proliferative response (compared with that caused by I region LD differences) and highly significant cytotoxicity against B10.A^{37,38}. The simultaneous presentation of LD and SD stimuli, however, leads to a markedly enhanced cytotoxic response against SD different target cells (LD-SD collaboration). The CML % is linearly related to the log of the number of CTLs; a difference from 21.8% to 40.4% thus represents much greater than a twofold difference in terms of lytic potential³². The LD-SD collaboration will be analysed in greater detail later (see Table 4). It should be pointed out, however, that whereas the stimulation of AQR cells by B10.T(6R) does not lead to CML directed at B10.A, relatively low level but highly significant CML directed at B10.T(6R) does result^{32,38} (see also Table 4).

These results demonstrate clearly the LD-SD co-operation; that is the presence of an LD difference in the stimulating mixture markedly enhances the development

Table 2 LD-SD collaboration in a three-cell experiment

³ H-TdR incorporated (c.p.m.) $\pm$ s.d.*	Target cell	%CML $\pm$ s.d.
10,842 $\pm$ 236 AQR + 6R _m	B10.A	-1.2 $\pm$ 3.1
4,113 $\pm$ 190 AQR + B10.A _m	B10.A	21.8 $\pm$ 2.1
8,292 $\pm$ 309 AQR + 6R _m + B10.A _m	B10.A	40.4 $\pm$ 2.8

*The control culture AQR(AQR)_m incorporated 2,926 $\pm$ 254 c.p.m. ³H-TdR.

The strain B10.T(6R) is abbreviated as 6R. The designations for the seven regions and subregions of *H-2* (K, I-A, I-B, I-C, S, G and D) for the strains are as follows: AQR, qkkddddd; 6R, qqqqq?d; B10.A, kkkddddd. As such, AQR and 6R are *H-2* SD identical but different by LD, and AQR and B10.A are K region different but identical for the rest of *H-2* (D. J. Schendel and F.H.B., unpublished data).

of cytotoxicity against the SD region target even though the LD stimulus by itself does not generate cytotoxic cells against that target. In contrast, the presence of the SD antigen does not enhance anti-LD (anti-I region) CML (see Table 4 and later discussion).

Experiments with human cells yield similar results with one exception: SD antigenic disparity "alone" (that is *HLA-D* identity) leads neither to a strong proliferative response nor to a detectable cytotoxic response^{25,29,30,36,39} contrasting with the MLC and CML associated with SD region differences in mouse. The high magnitude proliferative and cytotoxic responses seen in LD plus SD disparate human MLCs would suggest that this difference is not due to insensitivity of the human method although the culture technique cannot be ruled out as the explanation. The discrepancy could be based on variation of human and mouse SD antigen presentation or recognition. Alternatively, it may be explained by the evolutionary generation of somewhat different arrangements of the genes of human and mouse MHCs (see Fig. 1). In mouse, the *H-2K* locus and loci of the I-A sub-region are very closely linked; recombinational events between them may in most cases result in the inclusion of some relatively weak LD loci in what we call the "SD region". In contrast the probably tenfold greater recombinational frequency between *HLA-B* and *HLA-D* may prevent such LD loci from being included in the SD segment of most human recombinant chromosomes, thereby allowing a more complete genetic separation of LD and SD antigens in human recombinants.

Cellular recognition of MHC antigens

The cells responding in MLC and CML are primarily T cells. On the basis of evidence that functionally different subpopulations of T cells exist⁴⁰, we suggested in 1972⁴¹ that the LD-SD dichotomy might be explained at the cellular level by the existence of two separate subpopulations of T cells, one responding primarily to LD (the proliferating helper cell—PHC) and another responding to SD (the cytotoxic T lymphocyte—CTL). Results of experiments using cellular immunoabsorbants⁴² (monolayers of allogeneic adherent cells) were consistent with the existence of two separate populations of T lymphocytes, one responding to LD by proliferation and not adhering to an allogeneic monolayer; the other, the CTLs adhering to the monolayer. These two cell populations can be separated before sensitisation in MLC. An example of such an experiment is shown in Fig. 3. Cells of individual A are adsorbed on to either an A, B or C monolayer; 1 h later the non-adherent cells are removed from the monolayer and stimulated with cells of individual B and tested on B target cells. The level of cytotoxicity generated against B when the non-adherent A cells recovered from the B monolayer are stimulated with B_m is minimal compared with similarly stimulated non-adherent cells from the A or C monolayers. In 12 experiments⁴² the average reduction in cytotoxic activity

(lytic units or potency) of non-adherent lymphocytes after pre-adsorption on allogeneic monolayers was 70% whereas only a 12% reduction in ^3H -TdR incorporation measured in MLC was observed. The adsorption experiments are consistent with the finding that the CTLs divide (ref. 43 and B. J. Alter and F.H.B., unpublished) (incorporate ^3H -TdR), although they seem to contribute only a relatively small percentage to the total ^3H -TdR incorporated in a normal MLC—most is incorporated by the PHCs^{41,42}.

The concept of two cell populations responding to LD and SD antigens was strengthened by the findings of Cantor and Boyse^{44,45} using antisera against Ly cell surface antigens. These antigens are T-cell differentiation markers controlled by three loci, *Ly-1*, *Ly-2* and *Ly-3*. It seems

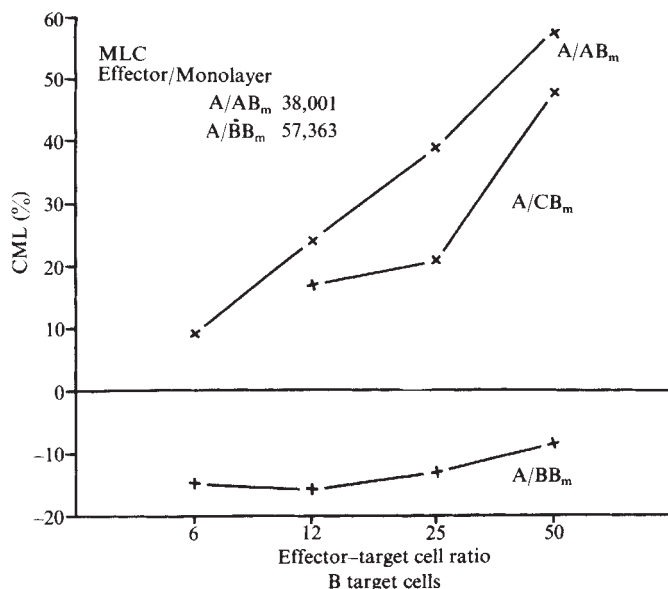


Fig. 3 Non-sensitised reciprocal adsorption. Cells of individual A (listed before the /) are adsorbed on either an autologous, A, monolayer or on allogeneic monolayers from individuals B or C (listed immediately after the /). The non-adherent cells from the monolayer are then stimulated with mitomycin-C treated cells from individual B (designated B_m), and assayed 5 d later for their ability to lyse B target cells. The marked reduction in cytotoxic activity following adsorption on the B monolayer, compared with the A or the C monolayer, is shown with no significant decrease in the proliferative activity. The reciprocal experiment stimulating the non-adherent cells with mitomycin-C treated cells of individual C and testing on C target cells in CML gave qualitatively similar results. (From K. S. Zier and F.H.B., unpublished.)

that LD responsive PHCs are *Ly-1*+ and do not mediate cytotoxicity; *Ly-2,3*+ cells divide and become CTLs. Support for the dual T-cell concept has also come from studies of Wagner *et al.*^{46,47}.

Responses to LD and SD *in vivo*

The functional distinction of LD and SD antigens has been supported largely by experiments involving discrimination of cellular immune recognition *in vitro*. Of great importance is the integration of both antigenic and cellular distinctions into the overall process of allograft reactions *in vivo*. The importance of LD and SD antigens has been studied *in vivo* using both graft versus host and graft rejection assays.

Essentially similar results have been obtained in a number of graft versus host (GvH) test systems including splenomegaly, lymph node enlargement, or increased radioactive IUDR incorporation into spleen or lymph node cells following injection of histoincompatible immunocompetent cells. Results closely parallel those found in the MLC; that is

LD differences are very potent at eliciting GvH reactions, the SD differences are relatively weak in this regard⁴⁸⁻⁵⁰. Representative results from one experiment performed by Elkins *et al.*⁵⁰ are given in Table 3. Most important are the findings that the LD differences (I+S) are quite strong in eliciting the response; two strains that differ by only the K region or the D region plus I-B, I-C, S and G do not elicit a significant response in this test system⁴⁹.

In man, the survival of transplanted tissue in non-prensensitised individuals is prolonged by identity for either LD or SD antigens⁵¹⁻⁵³. Of the two types of MHC determinants, preliminary data in man suggest that identity for LD has greater predictive significance for the length of graft survival, at least with respect to skin and kidney grafts⁵¹; but this distinction requires further study.

One important facet of the response to allogeneic tissue *in vivo* is not usually detected by routine MLC and CML testing *in vitro*: the influence of alloantibody. Human renal graft recipients immunised with multiple whole blood transfusions often produce cytotoxic alloantibody. The presence of antibody directed against donor tissue SD antigens induces accelerated and sometimes hyperacute

Table 3a Response of B10.T(6R) thymocytes to I+S region difference

Host strain	% activity recovered in spleen*	H-2 region difference
(AQR × 6R) F ₁	0.63 (0.44-0.73)	I+S
B10.A	1.12 (1.10-1.44)	K+I+S
B10.T(6R)	0.03 (0.02-0.03)	None

*Median (range)

Table 3b Response of B10.A thymocytes to various regions of the H-2 complex

Host strain	Number of hosts	% activity recovered in spleen*	H-2 region difference
AQR	6	0.03 (0.01-0.04)	K
B10.A(4R)	4	0.02 (0.02-0.02)	I-B, I-C, S, G, D
B10	6	0.36 (0.17-0.47)	Complete
B10.A	5	0.02 (0.01-0.03)	None

*Median (range).

The response of thymocytes to various regions of the H-2 complex is assayed by studying the incorporation of radioactive iodouracil-deoxyriboside (IUDR) into the spleens of recipient animals. The % activity recovered in spleen is in relation to the entire radioactivity injected. Control values usually range between 0.02 and 0.05%; a value of 0.36% is highly significant. (After Elkins *et al.*⁵⁰.)

rejection⁵⁴. Other transfused patients who do not have antibody directed at donor SD antigens more readily accept and retain allografts than non-transfused transplant recipients. van Rood has suggested this increased graft survival after many transfusions results from immunisation to LD determinants and subsequent production of an antibody that enhances graft survival (results presented at the annual meeting of the AASCT, 1975). Such enhancement has been demonstrated in mouse by passive transfer of anti-Ia antisera that prolong graft survival even when administered in $\mu\text{g kg}^{-1}$ quantities; anti-SD antibody did not enhance graft survival⁵⁵. The apparently differential role of

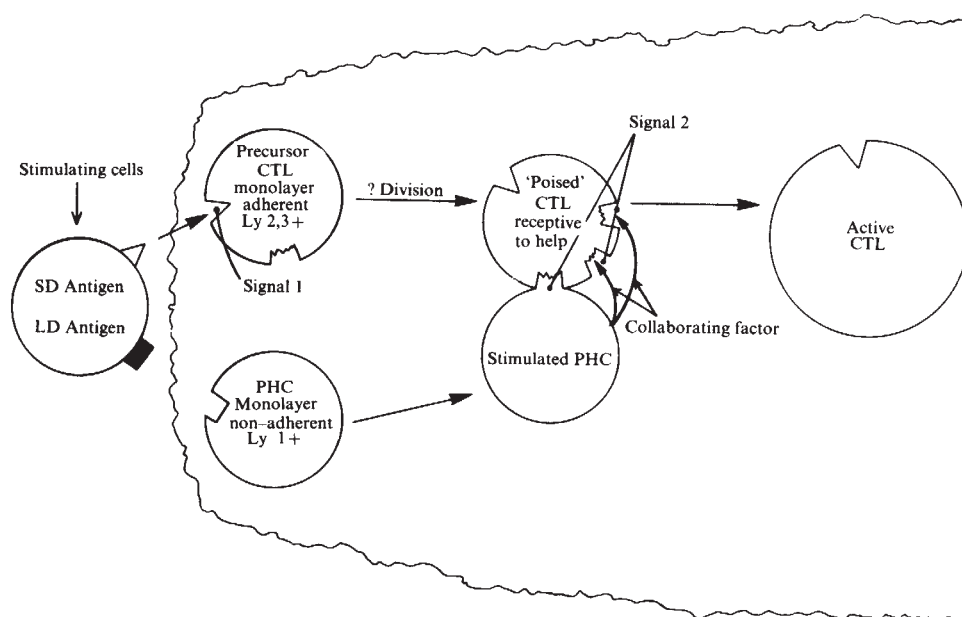


Fig. 4 Primary response. A stimulating cell carrying both an SD antigen(s) and an LD antigen(s) will evoke separate responses in two T-cell subpopulations in a manner similar to that depicted in the original cellular model proposed to explain the LD-SD dichotomy⁴¹. We can refer to the recognition of the SD antigen by the precursor CTL as signal 1. This cell is monolayer adherent and Ly-2,3+. It seems, on the basis of results shown in Table 4, that when the LD and SD stimuli are added at different times after initiation of culture, the SD antigens initiate a step in differentiation which may be independent of LD. This differentiation step results in a "poised" CTL which is receptive to help. A separate cell population of T lymphocytes, the proliferating helper cells, respond to LD antigens. This cell is monolayer non-adherent and Ly-1+. The help given by the PHC to the poised CTL might be delivered either by cell-cell contact between the PHC and the poised CTL or by a "collaborating factor" (secreted from the stimulated PHC) to which the poised CTL can respond. This is an obvious analogy to the model for T-B cell collaboration. It is a purely gratuitous assumption that the receptor on the CTL that recognises the collaborating factor is present in an effectively increased number on the cell as compared with the precursor CTL. Many alternative mechanisms may account for the fact that the poised CTL is more receptive to help than the precursor CTL. The direct contact between the stimulated PHC and the poised CTL or the collaborating factor would provide signal 2 to the CTL and lead to further differentiation to an active CTL. To what degree a CTL receiving only signal 1 can become cytotoxic and the other aspects of this model are discussed in this article. We have recently elaborated on this model to include the "secondary" responses of lymphocytes in culture⁵⁸.

antibody directed at Ia (?LD) and SD molecules once again is consistent with a dichotomy of function.

An attempt at synthesis

We have discussed above data supporting the contention that LD and SD antigens are functionally distinct and that two separable populations of T lymphocytes respond to these antigens. We shall now attempt to expand on our previous model to explain how LD and SD antigens and their respective responding lymphocyte populations interact in the generation of a specific cytotoxic response. The elaboration is based on the Bretscher-Cohn model for B-cell activation⁵⁶ and is in general agreement with Crichton and Lafferty⁵⁷. It would suggest that the CTL receives two signals for activation: "signal one", the SD antigen, and "signal two" given by the PHC after it has been stimulated by LD. It is quite likely that many stimuli will, either through the PHC or by acting directly on the CTL, be able to provide signal two. This model is illustrated in Fig. 4. In this context the LD-SD dichotomy can be summarised as follows. (1) The SD antigens function as the prime CTL activating determinants and as targets for these cells. The weak proliferative response stimulated by SD region differences in mouse may reflect CTL division or stimulation of an SD reactive PHC population. Alternatively the proliferative response is due to PHCs responding to LD determinants included in the "SD region difference". (2) A strong MHC LD locus, separable from the SD loci, is primarily responsible for stimulating the strong proliferative response in MLC and presumably for inducing the PHC-CTL helper effect. One or more weaker MHC LD loci probably exist. Weak but significant cytotoxicity is often associated with I region differences; it is not established

whether this is directed against LD as opposed to other I region antigens. (3) There is very substantial and clear evidence for LD-SD collaboration based on both genetic and cellular studies. The presence of an LD stimulus in the sensitising MLC markedly enhances the development of cytotoxicity aimed at the SD region targets unless maximum CML is already present (M. B. Widmer, F.H.B., H. R. McDonald and J. C. Cerrotini, unpublished) *in vitro* due to the presence of a strong signal 2 such as may be provided by heterologous serum or conditioned medium, even though stimulation by LD alone does not generate cytotoxicity against the SD target. It is not known whether the LD helper effect is essential and the CML generated in SD region different strains is due to an LD-like stimulus determined by gene(s) in that region, or that LD simply influences the quantitative amount of CML induced by SD alone. The suggestion that the SD antigens actually initiate (signal 1) a differentiative response that is expanded, or allowed to differentiate further, by the presence of cells responding to the LD stimulus (signal 2) is based on kinetic experiments in which SD and LD stimuli are added to responding cell populations at different times. The basic findings⁵⁸, which look quite similar to those on T-B cell collaboration, are illustrated in Table 4. If the SD different stimulus (in a "three-cell" protocol) is added at time zero and the LD stimulus is delayed for 24 h, the CML against B10.A target cells is in fact increased, although the kinetics of the development of the cytotoxic response aimed at the SD antigens may differ (see day 5). Adding the 6R_x cells 48 h after initiation of the AQR(B10.A)_{11V} culture still results in 48.7±4.8% CML on B10.A target cells. In the reciprocal direction, delay in adding the SD different cells, after adding the LD different cells on day zero, results in a similar delay in the development of CML. We have

discussed this in detail elsewhere³⁸. Most important, the presence of the SD stimulus does not seem to potentiate "anti-LD" CML. This is in contrast to the potentiation of anti-SD CML by an LD stimulus, and further supports the LD-SD dichotomy.

Two findings might seem to contradict the need for signal 2 in the generation of CTLs. First, that Ly-2,3+ lymphocytes alone can respond to an entire MHC difference and become CTLs⁴⁹. This apparent circumvention of the PHC role could be explained by several models. First, a relatively weak signal 2 can be given directly to the CTL by mechanisms other than PHC collaboration either by the SD antigens themselves or some other stimulus. Second, the Ly-2,3+ cells may be a heterogeneous population, a portion of which can respond to the LD antigens and thereby provide the helper effect to allow CTLs within that population to develop. This is consistent with the finding in those same studies that an Ly-2,3+ population depleted of Ly-1+ cells still shows a highly significant proliferative response to strains differing for only the I and S regions (that is, LD different-SD identical strains) without showing CML activity⁴⁵. The lack of cytotoxicity in this situation suggests that the proliferation seen is not due to division of CTLs. Third, the Ly-1+ cells that proliferate in response to LD (proliferating "helper" cells) may not be required for the induction of CTLs but may simply expand an ongoing response and may be functionally distinct from Ly-2,3+ LD reactive cells. Finally, it may be that PHCs in the stimulating cell population respond to LD antigens on the responding cells and provide the helper effect to the responding CTLs. If help is provided by means of a factor, the observation that mitomycin-C treated cells do produce factors is important⁵⁰.

The second apparent contradiction is the finding that an SD region difference alone can stimulate both proliferative and CML responses in mouse. Ultraviolet irradiation of stimulating cells abrogates their ability to evoke a proliferative MLC response thus appearing to ablate LD expression; the SD antigens on ultraviolet-treated cells can still be detected serologically⁶⁰. In Table 4 the SD region different stimulating cells are treated with ultraviolet irradiation rather than mitomycin-C³⁸. In these circumstances the cells evoke minimal if any proliferative or cytotoxic response. The addition of an LD stimulus to the sensitising mixture however still leads to a very marked cytotoxic response against the SD different target cells.

The three-cell experiments with ultraviolet-treated stimulating cells show that an SD stimulus that is effective at activating CTLs is not capable of strongly stimulating the PHC and does not by itself lead to as great a cytotoxic reaction as in the presence of LD. At least four mechanisms could be invoked to explain the difference between the mitomycin-C treated (see Table 2) and ultraviolet-treated SD region disparate stimulating cells. First, the same determinant on the SD molecule may be responsible for activating the PHC and CTL but the two cell populations have different requirements for activation (possibly due to molecularly different recognition molecules). Treatment with ultraviolet light leaves a cell still capable of activating CTLs but not PHCs. For example, the cell surface density of SD antigens may be reduced by ultraviolet treatment making the SD stimulus ineffective at stimulating PHCs or providing signal 2 directly to the CTL. Second, cellular contact initiated by recognition of surface SD antigen might allow a cell membrane interaction (possibly unrelated to antigen recognition) to provide a weak substitute for signal 2. Ultraviolet-treated stimulating cells provide the SD stimulus (signal 1) but are unable to provide the substitute signal 2 and therefore require an additional generation of signal 2 provided by the LD stimulus by way of PHCs. Third, an ultraviolet-sensitive part of the SD molecule different from that triggering the CTL may activate the PHC. This is similar to our initial analogy of a carrier-hapten system to the LD-SD problem^{15,35} and the reiteration of that model applied to the SD antigen⁶⁵. Fourth, there may be LD-type loci in the K region separable from the SD loci³². Conceptually, in terms of phenotypic expression, the third and fourth models both suggest that the LD and SD antigens are determined by genetically separate sites; the question whether the LD and SD stimuli are determined by separate genes or separate subclonistic segments is not terribly important in this context.

Mutants of H-2

The studies that first led to the definition of H-2 LD loci^{15,16,62} were done using a mouse strain, discovered by Bailey, that carries a spontaneous mutation in *H-2* to the left of *Ss*⁶³. The phenotypic expression of the mutation is reciprocal skin graft rejection between the parental strain, C57BL/6, and the mutant, H(zl), in spite of apparent serological identity of cells of the two strains. More recent

Table 4 Interaction kinetics

Effector combination	MLC ³ H-TdR incorporation (c.p.m. $\pm$ s.d.) day 5	Target cell	% CML (mean $\pm$ s.d.)	
			Day 5	Day 6
AQR + 6R _x + B10.A _{UV}	25,802 $\pm$ 1,090	AQR	-0.57 $\pm$ 1.5	-5.8 $\pm$ 2.8
		6R	13.6 $\pm$ 2.1	17.5 $\pm$ 5.9
		B10.A	35.9 $\pm$ 3.5	50.0 $\pm$ 4.4
AQR + 6R _x	26,184 $\pm$ 1,057	AQR	-1.7 $\pm$ 1.1	-10.4 $\pm$ 3.2
		6R	15.1 $\pm$ 1.9	18.8 $\pm$ 3.2
		B10.A	-0.6 $\pm$ 2.0	-4.1 $\pm$ 4.7
AQR + B10.A _{UV}	1,520 $\pm$ 104	AQR	-3.2 $\pm$ 0.9	-12.5 $\pm$ 2.0
		6R	-2.3 $\pm$ 1.8	-12.3 $\pm$ 2.5
		B10.A	1.5 $\pm$ 1.7	8.1 $\pm$ 2.8
AQR + B10.A _{UV} + 6R _x (24 h)	27,863 $\pm$ 462	AQR	-0.5 $\pm$ 0.8	-7.5 $\pm$ 2.0
		6R	-0.6 $\pm$ 2.2	11.2 $\pm$ 3.0
		B10.A	5.2 $\pm$ 1.7	70.0 $\pm$ 7.9
AQR + 6R _x + B10.A _{UV} (24 h)	38,224 $\pm$ 1,339	AQR	-0.5 $\pm$ 0.5	-3.5 $\pm$ 2.0
		6R	13.9 $\pm$ 1.8	14.9 $\pm$ 4.2
		B10.A	12.1 $\pm$ 1.6	40.3 $\pm$ 7.1
AQR + AQR _x	975 $\pm$ 34	AQR	-2.1 $\pm$ 0.9	-11.1 $\pm$ 2.7
		6R	-1.5 $\pm$ 1.7	-12.2 $\pm$ 3.6
		B10.A	—	8.3 $\pm$ 3.0

The control effector combination, AQR + AQR_x, gives 8.3 $\pm$ 3% CML on the B10.A target cells. It is thus difficult to know whether there is any significant killing on B10.A target cells when AQR cells are stimulated with B10.A_{UV}. A 24-h delay in adding the B10.A_{UV} cells results in a level of cytotoxicity on B10.A target cells on day 6 not significantly different from that found on day 5 when the LD and SD stimuli are added together at time zero. (D. J. Schendel and F. H. B., *Eur. J. Immun.*, in the press.)

studies suggest that a serological difference may exist^{64,65}. Consistent with this possibility are recent findings that the SD antigenic products of BL/6 and H(zl) differ by peptide mapping (J. L. Brown and S. Nathanson, personal communication). In MLC and CML, both a proliferative response and a very marked cytotoxic reaction were seen reciprocally (see Table 5); the magnitude of the cytotoxic response between cells of the parental and mutant strain is comparable to that observed in the responses between mouse strains known to differ for both LD and SD antigens, and significantly greater than that observed between strains differing only for an SD region. The existence of both the MLC proliferative response and CML cytotoxicity related to these mutants has been extensively discussed^{24,62,64,65}.

It is possible that the spontaneous mutants carry "point mutations" that affect only a single phenotypic product of the MHC, the SD molecule. By this assumption one must explain why CML equal to that found in LD+SD different strains is present in the C57BL/6-H(zl) combination. Two considerations may be helpful. The basis on which these mutants were found, that is relatively rapid skin graft rejection, may have selected for highly immunogenic SD antigens that can alone account for these findings. In addition, proliferative responses between C57BL/6 and the mutants are on the average weaker than those seen in H-2 disparate strains. This would again be consistent with a highly immunogenic SD difference that by itself induces a sufficient helper proliferative response (or in some other way gives signal 2) to allow strong CML to develop. We do not know how great the helper response must be for CML to develop, and a relatively low response may in this case be enough. The discussion above of the apparent circumvention of LD-SD collaboration in SD region different strains alone is also applicable in this instance.

The spontaneous derivation of H(zl) does not prove that the mutant differs only for an amino acid substitution in the SD gene product. Subsequent skin graft screening of C57BL/6 animals revealed a surprisingly large proportion (more than 1%) of independently derived H-2 mutants⁷⁸ suggesting a somewhat non-conventional mutation mechanism. Because F₁ hybrids of any two of these H-2 mutants reject parental skin grafts, it seems that at least one common genetic determinant was altered in each independent mutational event; however, these "complementation" studies do not exclude the possibility that the mutational event also resulted in changes of other MHC controlled determinants. It does not seem unreasonable to hypothesise that a spontaneous mutation would affect the phenotypic expression of more than a single molecule. Several mechanisms could be suggested.

First, it may be that spontaneous mutation affects only one molecule in terms of an amino acid substitution, for instance the SD antigen. That SD antigenic molecule may interact in a physically intimate manner with the LD antigen so that a change in the SD antigenic molecule may result in a change in the expression of the LD antigen. We have recently discussed possible LD-SD epistasis in MLC reactions⁶⁶. Arguing against this possibility is the lack of any such finding in mouse strains carrying recombinant chromosomes. Second, it may be that these mutant strains were generated by the effects of complex mutation events. By this model we predict that the LD antigens, of these mutant strains, when isolated, will have one (or more) amino acid substitution(s) from the parental LD, as does the SD molecule in the case of H(zl). Third, it may be that mutations of LD and/or SD products are not infrequent and are, in fact, carried by many inbred lines. Let us hypothesise that rapid skin graft rejection between the two strains is dependent on differences for both LD and SD type antigens. The mutants mentioned here were identified by skin grafting only; the parental mice were not routinely screened for mutations in LD alone, as detected in MLC,

Table 5 MLC and CML studies in the H-2 mutant H(zl)

<i>a</i> , MLC data		
Responding cell	Stimulating cell	³ H-TdR incorporation (c.p.m.)
B6*	B6 _x	3,409 ± 962
B6	H(zl) _x	36,665 ± 5,310
B6	7R _x *	40,620 ± 1,507
H(zl)	H(zl) _x	2,001 ± 974
H(zl)	B6 _x	24,677 ± 7,697
H(zl)	7R _x	68,727 ± 14,468
<i>b</i> , CML data		
Responding cell (effector)	Stimulating (sensitising) cell	Target cells
		B6 H(zl) S*
B6	H(zl) _x	5.89 ± 5.01† 89.43 ± 5.15 9.79 ± 3.00
B6	S _x	-0.15 ± 2.05 23.72 ± 9.08 55.95 ± 6.94
H(zl)	B6 _x	57.45 ± 14.13 -0.26 ± 4.13 0.76 ± 1.92

*B6 = C57BL/6By; S = B10.S; 7R = B10.S(7R).

†% CML ± s.d.

(From M. Widmer, unpublished.)

or for mutations in SD alone, as detected in "three-cell" CML protocols. It is thus possible that some of the parental BL/6 lines may have been carrying either an LD or an SD mutation that was not detected by skin grafting. Subsequent mutation could then yield "spontaneous" mutants identified by skin grafting because they now differed from the original parental line for both LD and SD antigens.

Any one of these explanations as well as other models would explain the complex phenotypic expression in these mutants; the biochemical isolation and characterisation of LD and SD phenotypic products will be of tremendous benefit in understanding the LD-SD dichotomy, particularly with respect to these mutants. Clearly, the LD-SD model is an oversimplification of the genetics of histocompatibility antigens within the MHC; given our present state of knowledge, the model of a functional dichotomy of LD and SD antigens is consistent with all data collected so far, including these mutants, and serves as an heuristically valuable model.

Questions of genotypic identity

Responses of T lymphocyte subpopulations have been ascribed to antigens associated with a given H-2 or HLA region. In considering the genetic relationship of these antigens and the other functions ascribed to the same MHC (sub)regions, three basic questions of identity arise: (1) are the target antigens recognised by the CTLs identical to the SD molecules or, even more specifically, the determinants on the SD molecules that are recognised by antibody? (2) Are the LD antigens identical to the Ia antigens? (3) Are the LD antigens identical to the products of the I region locus^{18,61} causing skin graft rejection?

We and others have in the past discussed whether one can equate SD antigens with CTL target determinants^{24,62,67,68}. Data presented in Table 6 demonstrate that human CTLs sensitised to lyse cells from the stimulating cell donor can also lyse target cells from a third party not sharing any serologically detectable cross reactivities with the stimulating cell donor. Experiments using a CML "blocking" technique have demonstrated that this cross killing is specific⁶⁸; cytotoxicity of target cells from a third party results from CTL recognition of shared antigens on the initial stimulating cells and the target cells. Family studies have demonstrated⁶⁹ that these serologically undetected shared antigens are genetically controlled within the MHC.

Table 6 CML cross killing

CTLs	W	Target			Z
		X	Y		
ZW _m	62.5±2.8 (25)	55.9±3.2 (10)	38.5±2.7 (2)		1.7±3.9
ZX _m	48.4±4.3* (10) ^b	67.6±6.2 (25)	29.3±3.5 (1)		0.3±3.1
ZY _m	40.7±3.8 (6)	28.6±2.3 (2)	58.9±3.4 (25)		-1.6±3.2
Z--	0.4±1.7	-3.1±2.3	-1.7±2.5		—

CTLs from the indicated mixed leukocyte cultures as well as unstimulated cells from Z, were tested on target cells from individuals W, X, Y and Z. No cross reacting or shared antigens were detected serologically among individuals W, X and Y. Typing results are as follows: W = (1, 8); X = (2, 12); Y = (10, 7, 27); Z = (10, 11, W15, W16). (In other experiments similar results were obtained with all four antigens of HLA-A and HLA-B defined in every individual.) Percentage cytotoxicity by 25×10^4 CTLs on 1×10^4 target cells are shown. Potency values in parentheses represent the number of specifically sensitised cells from Z that cause the same percentage cytotoxicity as 25×10^6 of the CTLs being examined. Potency values were interpolated from a log-linear graph of several specific CTL concentrations against percentage cytotoxicity. Control values for each target (^{51}Cr c.p.m.): W, S.R. = 211 ± 20 , Max. = $1,913 \pm 22$; X, S.R. = 206 ± 16 ; Max. = $1,089 \pm 46$; Y, S.R. = 246 ± 30 ; Max. = $1,597 \pm 50$; Z, S.R. = 322 ± 42 , Max. = $1,714 \pm 72$. (From ref. 68.)

These data could thus be interpreted to suggest that the target for the CTLs is determined by MHC genes that are different from those determining the SD antigens, a suggestion made in our initial study with the C57BL/6-H(2l) mutant combination⁶². Alternatively, the cytotoxic target antigen may be the SD molecule itself, that is the same molecular determinant that evokes antibody production is recognised by CTLs; the antibody-producing B cell and the CTL, however, recognise the same determinant with different specificity, possibly by molecularly distinct receptor molecules, as suggested recently⁶⁷. It is also possible that the CTLs recognise a different portion of the SD molecule from that recognised by B-cell produced antibody. The recent findings in mouse that CTLs may react with not only the private but also the public antigens of H-2 SD molecules may provide an explanation⁷⁰; in man it is unlikely that antisera detecting public HLA SD antigens are frequently used. There is direct evidence that the CML targets are not the SD molecules^{71,72}.

Evidence suggesting at least partial identity of Ia and LD determinants⁷³ demonstrated that anti-Ia antisera blocked the stimulating determinants in an MLC⁷⁴. These findings can be used to argue that LD and Ia are, at least in part, identical; the ability of many different antibodies to block MLC reactions, however, prevents the firm acceptance of this last conclusion. In human studies, it has been noted by van Rood and his colleagues that there seem to be exceptions to the reaction patterns of the anti-human Ia antisera and the presumed LD determinants carried by those cells⁷⁵. Even if LD antigens can be so defined, we would stress the difficulties of equating Ia antigens (detected by antibody) with LD antigens (detected by T cell reactivity in MLC). This caution is emphasised by the results we have discussed above in considering whether the antigen recognised by CTLs is the same SD antigen detected by alloantisera. In fact, there is no critical evidence that LD "determinants" are expressed on the cell surface.

A locus in the I region leads to skin graft rejection^{48,61}. It might be reasonably argued that this locus is identical with the LD locus. One would then have to explain why LD-like differences in other parts of the H-2 complex are not associated with skin graft rejection. At least two possible explanations exist. It may be that I-A sub-region LD antigens collaborate with other I-A determined antigens (perhaps Ia) that are the targets for the CTLs; no such targets may exist in association with LD antigens in other regions. Alternatively, the LD antigens are the targets for the rejection response; the reason why skin graft rejection

may only be associated with LD differences in the I-A subregion is because these are the strongest LD differences of H-2. A threshold level of incompatibility may be needed before skin graft rejection will proceed.

Summary

We have emphasised the functional dichotomy of MHC LD and SD antigens as well as the differences in cellular responses to these antigens. Perhaps in so doing we have failed to stress adequately the similarities that exist. But while the similarities (for example skin graft rejection associated with both K and I region differences) are so very clear, the differences have best allowed our progressive understanding of MHC induced cellular responses from the perspective stressed in this article. Of greatest importance to our understanding of these transplantation antigens are the potentially differential roles for the LD and SD antigens in the complex series of events that are collectively referred to as the "allograft reaction". It has been suggested that these differences may be "merely quantitative"⁶¹. This possibility has been discussed repeatedly in our previous reports on the distinction of LD and SD. In fact, the great bulk of biological phenomena can be reduced to quantitative differences. It would seem to us that sufficient evidence for such differential activity exists to make the LD-SD dichotomy model an heuristically valuable one for purposes of designing future experiments. We have discussed the clinical relevance of this model elsewhere²³.

Many authors have speculated^{41,76,77}, and evidence has been gathered^{19,20} to suggest, that cell surface antigens associated with the MHC are important in developmental and other cell interactions. Some studies have directly addressed the question of the need for MHC compatibility to allow cell interaction to proceed optimally. It thus seems most appropriate that the genetic complex with which we are dealing has been termed the major histocompatibility complex; allowing for the literal interpretation of this term this may be the genetic region that by its influence on "tissue compatibility" may control critical cellular interactions in addition to those observed in allograft reactions. It is the simple good fortune for those whose attention was focused on this complex by transplantation problems to find themselves with a panorama of biological phenomena that require extensive experimental probing and integration, hopefully ultimately leading to an understanding of the MHC in a broader context than has to date been possible.

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articles

Two origins of replication in composite R plasmid DNA

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Electron microscopic examination of a composite R plasmid made up of an RTF component and an r-determinants component shows that replication may originate from either the RTF origin or the r-determinants origin of replication.

MANY of the drug resistance plasmids (R plasmids) of the Enterobacteriaceae are composite structures consisting of two distinguishable components: a resistance transfer factor (RTF) which mediates the transfer of these plasmids during bacterial mating and an r-determinants component which harbours most of the drug resistance genes of the plasmid¹⁻³. Each of these components is capable of autonomous replication in certain host strains of bacteria; that is, each is a "replicon"¹⁻⁶. In most genera of the Enterobacteriaceae the RTF and r-determinants components are united to form the composite R plasmid structure^{1,2,4,6-8}. This is also true in *Proteus mirabilis* when this host strain is cultured in drug-free medium^{7,9,10}. When *P. mirabilis* is cultured in medium containing any of the drugs to which the r-determinants confer resistance, R plasmids are formed

which consist of a single copy of the RTF and multiple, tandem copies of the r-determinants component (poly-r-determinant R plasmids)^{2,5-7,9,10}. Autonomous poly-r-determinants consisting of multiple copies of the r-determinants component are also formed⁹. Since both the RTF and the r-determinants component must contain an origin at which replication is initiated, the replication of R plasmids in *P. mirabilis* provides a model system for studying the replication of chromosomes containing multiple origins of replication. Here we show that either of the two origins of a composite R plasmid can be used for the initiation of R plasmid replication.

We have previously shown that there is a substantial increase in the fraction of replicating R plasmid DNA in *P. mirabilis* cells as a consequence of decreasing the rate of DNA chain elongation by limiting the concentration of DNA precursors^{10,11}. Using this technique, three experiments were carried out to isolate replicating molecules of R plasmid NR1 DNA from *P. mirabilis*. In the cell culture conditions used, the R plasmid DNA exists as the undissociated composite structure (circular contour length of 37 μ m; molecular weight 63×10^6) which consists of a single copy of the RTF (29 μ m; molecular weight 49×10^6) and a

single copy of the r-determinants component ($8.3 \mu\text{m}$; molecular weight 14×10^6)^{7,9-11}. In two of the experiments thymine limitation was used to increase the fraction of replicating R plasmid DNA; in the third experiment treatment of the cells with hydroxyurea was used¹¹. Fractionated R plasmid DNA was spread for electron microscopy at pH 10.7 so that approximately 20 small denaturation sites

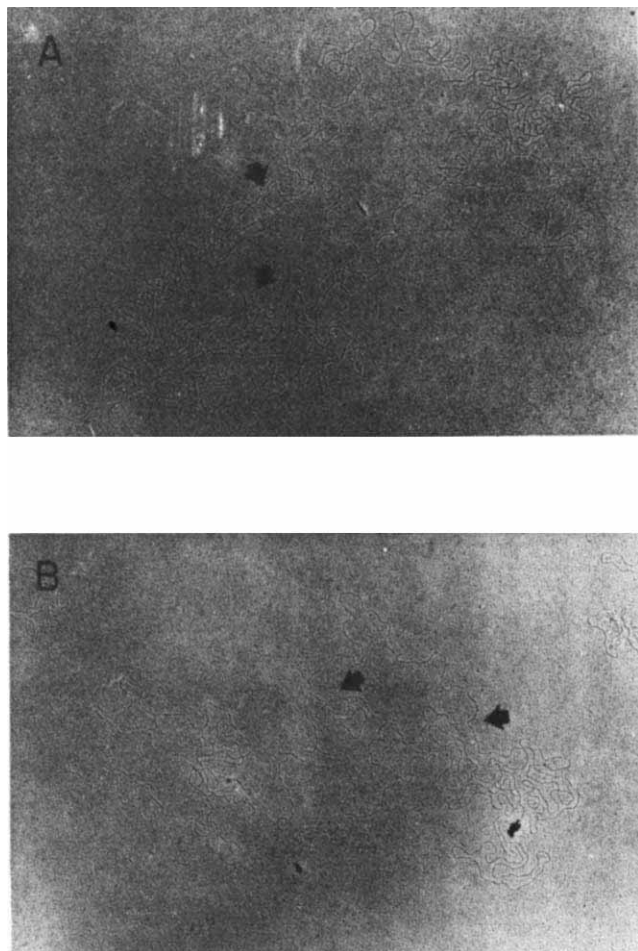


Fig. 1 Two examples of replicating molecules of composite R plasmid NR1 DNA. The two arrows indicate the two branch points (replication forks) of the replicating molecules. These two replicating molecules were isolated from R⁺ *P. mirabilis* cells after addition of hydroxyurea to the growth medium. One molecule has initiated replication in the RTF component (A) and the other in the r-determinants component (B) of the composite R plasmid DNA. These molecules are designated as H-A and H-B in Fig. 2.

would be present in the R plasmid DNA¹². We could thus compare the distribution of denaturation sites in the replicating molecules with the denaturation map of composite NR1 DNA. This made possible the orientation of the replicating molecules in a unique manner. The orientation of the replicating molecules was greatly facilitated by locating the $8\text{-}\mu\text{m}$ r-determinants region which has very few denaturation sites when the DNA is spread for electron microscopy at pH 10.7 (ref. 12).

The replicating NR1 DNA molecules observed in the electron microscope were double-branched (θ -type) circular structures when, as will be discussed subsequently, either the RTF origin (Fig. 1A) or the r-determinants origin (Fig. 1B) was used for the initiation of replication. The replicated regions (daughter branches) of the molecules were found to have equal contour lengths. Although

approximately 5% of the molecules observed in the electron microscope contained branch points (replication forks), only about one-fourth of these was suitable for detailed analysis. The criteria for choosing replicating molecules for mapping were as follows: (1) at least 10, and as many as 20, denaturation sites in each molecule were preferred for the most accurate alignment of the molecule with the NR1 DNA denaturation map; (2) the locations of the two replication branch points were clear and unambiguous; (3) there was no ambiguity in following the contour of the molecules throughout their entire length.

In Fig. 2 the distribution of denaturation sites and the replication branch positions in aligned molecules of replicating R plasmid DNA are superimposed on the composite NR1 DNA denaturation map. For convenience of presentation, the circular NR1 DNA molecules are represented by a linear denaturation map. The r-determinants component is located to the left side of the map (0.0–0.23) and the RTF component to the right side (0.23–1.0)¹². Replicating molecules from the two thymine limitation experiments and the hydroxyurea experiment are designated T1, T2, and H, respectively. The weight-average histogram of the distribution of denaturation sites in all of the replicating molecules shown in Fig. 2 is presented in Fig. 3a. This histogram agrees well with the weight-average histogram (denaturation map) previously determined for non-replicating NR1 DNA molecules¹² (Fig. 3b).

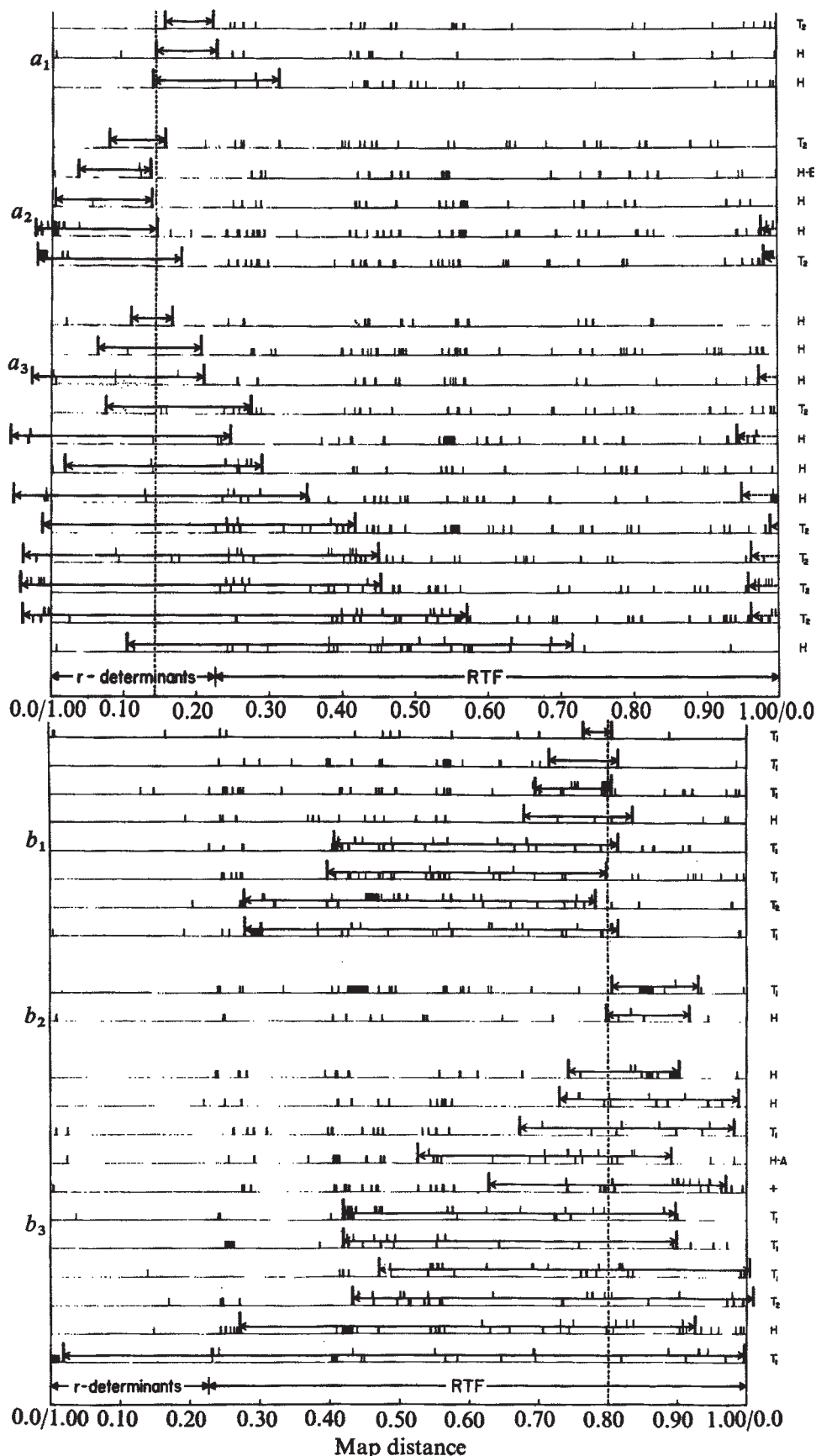
The extent of replication and the branch positions of replicating R plasmid molecules are designated by arrows in Fig. 2. The positions of the replication branches are consistent with two different origins at which replication of the composite R plasmid DNA can be initiated. One is located in the r-determinants component at a position of 0.14 on the denaturation map. The other is located in the RTF component at a position of 0.80 on the denaturation map. The replicating molecules with the shortest replication branches were consistent with only these two origins and helped to localise their positions on the map.

Replicating molecules isolated from the same culture of cells were found to have initiated replication at either of these two origins of replication. In the case of either origin, replication could be either bidirectional or unidirectional in either sense. The replication branch points were usually not symmetrically arranged about either origin of replication. Replicating molecules have not been observed in which both origins were used simultaneously (that is, with four branch points). Such molecules may, however, be much more likely to fragment during isolation and handling of the DNA which would diminish their proportion in the population of replicating molecules.

A single replicating molecule has been observed by chance in an exponential culture which had not been subjected to substrate limitation. The replication of this molecule (designated as + in Fig. 2b) was initiated at the RTF origin and replication was bidirectional.

In many replicating molecules the branch point to the left of the r-determinants origin and the branch point to the right of the RTF origin tend to cluster around the position 0.95–1.00 on the denaturation map, suggesting that this position could be a terminus of replication. For several reasons it seems unlikely that this position is a third origin of replication. First, the positions of the two branch points of all of the replicating molecules which were analysed are consistent with either the r-determinants origin or the RTF origin. Second, none of the molecules with a small extent of replication (less than 15%) was consistent with an origin at 0.95–1.00. The existence of a terminus of replication at 0.95–1.00 could explain the asymmetry in the distribution of the replication branch points about the two origins since the arrival of one of the replication forks at the terminus would interrupt replication in that direction.

Fig. 2 Denaturation sites and replication branch positions in aligned molecules of replicating R plasmid NR1 DNA superimposed on the composite NR1 DNA denaturation map. A thymine auxotroph of *P. mirabilis* harbouring the composite R plasmid NR1 was cultured in drug-free M9 minimal medium containing 0.06% casamino acids and other supplements¹¹. In two of the experiments (T1 and T2) exponential phase cells were filtered, washed, and resuspended in similar medium containing a limiting concentration of thymine ($0.05 \mu\text{g ml}^{-1}$). In a third experiment (H) a final concentration of 0.075 M hydroxyurea was added to the culture medium. Both substrate limitation conditions reduce the rate of DNA synthesis about 20-fold. After about a 50% increase in cell mass during substrate limitation, DNA was prepared from the cells. Replicating R plasmid DNA molecules were fractionated by caesium chloride preparative density gradient centrifugation¹¹. Fractionated R plasmid DNA was spread for electron microscopy at pH 10.7. Replicating, circular NR1 DNA molecules which had on the order of 20 small denaturation sites, were photographed and analysed¹². The denaturation sites are represented by the smaller vertical bars of varying width, the width of the bars being proportional to the size of the denaturation site. From the distribution of denaturation sites on the replicating molecules, the molecules were aligned relative to the composite NR1 DNA denaturation map¹². All the replicating molecules shown here are circular structures which have been superimposed on a linear composite NR1 DNA denaturation map for convenience of presentation. The extent of replication and the replication branch positions of individual molecules are designated by the arrows between the two larger vertical bars. Since the replicating molecules are circular, the arrows representing the replication branch positions have been extended through the 0.0/1.00 junction of the denaturation map to avoid interruption. In these cases, the extended part of the arrows is repeated as a dashed arrow at the corresponding region at the other end of the denaturation map. Molecules whose replication branches are consistent with the *r*-determinants origin of replication are shown in the group of molecules included in *a* and molecules consistent with the RTF origin are shown in the group of molecules included in *b*. The molecules in each of the classes *a* and *b* are divided into three subclasses depending on whether replication is unidirectional to the right (*a*₁ and *b*₂) or unidirectional to the left (*a*₂ and *b*₁); replicating molecules showing bidirectional replication are included in *a*₃ and *b*₃.



All 10 replicating molecules in the first thymine limitation experiment (T1 molecules) were found to initiate replication at the RTF origin. In the case of the T2 replicating molecules and the H replicating molecules, however, about three-quarters of the molecules used the r-determinants origin and only a quarter the RTF origin. Since only a relatively small number of molecules were examined in each experiment, the data do not give a statistically accurate value for the frequency of use of

in eukaryotic chromosomes using electron microscopy²⁰ and autoradiography²¹, indicating that there are multiple origins of replication. Poly-r-determinant R plasmids^{5-7,9,10} must also contain multiple origins of replication, the number depending on the number of copies of r-determinants. Analysis of replicating poly-r-determinant R plasmid DNA by denaturation mapping should make it possible to determine whether there is any specificity involved in the selection of an origin for the initiation of

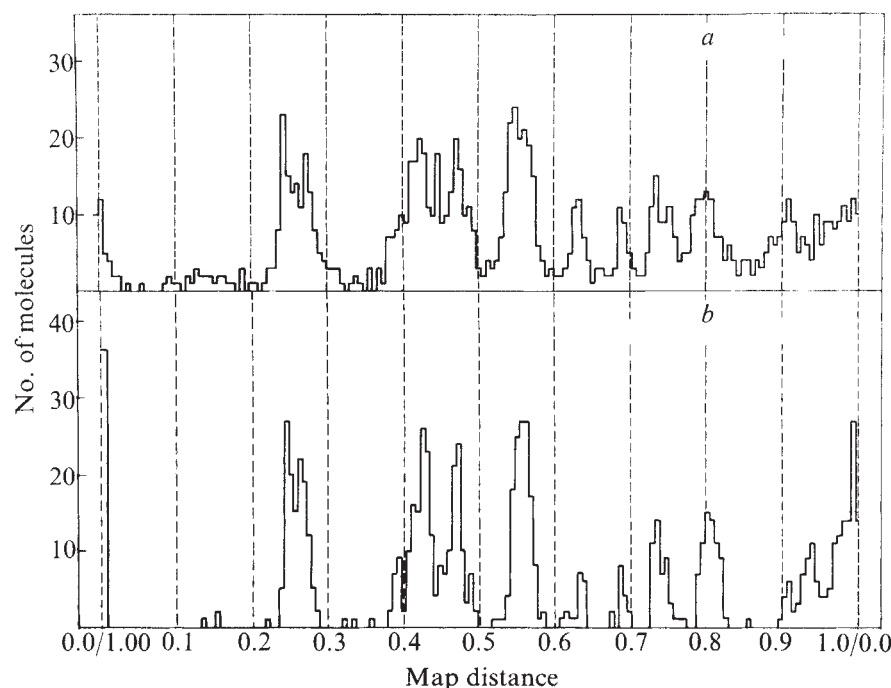


Fig. 3 Weight-average histogram of the distribution of denaturation sites in aligned replicating molecules of composite R plasmid NR1 DNA. *a*, Histogram constructed from the distribution of denaturation sites in the replicating NR1 DNA molecules shown in Fig. 2. The histogram includes all the denaturations present in the replicating molecules, even those present in the sister replication branches of the same molecule. *b*, Denaturation map of non-replicating composite R plasmid NR1 DNA¹².

either origin for the initiation of replication. Other experiments in which the total replicating plasmid DNA has been analysed (rather than individual replicating molecules in an electron microscope) also indicate that there is preferential use of the r-determinants origin. These experiments involve DNA-DNA hybridisation and buoyant density analysis of replicating R plasmid DNA and Agarose gel analysis of the fragments of replicating R plasmid DNA produced by treatment with restriction endonuclease *EcoRI* (D.P. and R.R., in preparation).

Our experiments have shown that there are at least two functional origins of replication in composite R plasmid NR1 DNA. The use of both origins is of course not mandatory since one origin might be dominant to the other in the initiation of replication. For example, the bacterial chromosome origin is dominant to the F factor origin in an Hfr strain¹³ or to a prophage origin in a lysogenic strain¹⁴ of bacteria. It is presently not known what determines the selection of either of the two origins for the initiation of composite R plasmid replication. It is possible that the substrate limitation technique itself could affect the selection. Since R plasmid NR1 replication is arrested when protein synthesis is inhibited^{6,15}, it will be of interest to determine whether there are specific R plasmid initiator proteins for each of the two origins or whether the same initiator proteins can function at either of the two origins.

Like phage λ DNA¹⁶, R plasmid DNA is replicated either bidirectionally or unidirectionally in either sense. Colicin E1 plasmid DNA, on the other hand, replicates unidirectionally from a unique origin¹⁷⁻¹⁹. Thus, in the two examples of plasmid replication which have been examined, different modes of replication have been observed.

Several internal replicating regions have been observed

replication, whether the additional r-determinants origins on poly-r-determinant R plasmids will influence the frequency of selection of the r-determinants origin relative to the RTF origin, and whether multiple initiations can occur on the same replicating R plasmid DNA molecule.

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Replication control in a composite plasmid constructed by *in vitro* linkage of two distinct replicons

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Although it carries two competent replication systems, a composite plasmid formed in vitro by linkage of the complete ColE1 and pSC101 plasmid replicons at their unique EcoRI endonuclease cleavage sites normally uses only the replication origin and functions of the ColE1 component. Restriction of ColE1 replication functions by DNA polymerase I deprivation results, however, in exclusive use of the pSC101 replication origin. When using the ColE1 replication system the composite plasmid is nevertheless incompatible with both the parent replicons. This suggests that a trans-dominant gene product is involved in plasmid incompatibility and supports negative control rather than positive control models for regulation of the initiation of DNA replication.

INITIATION of DNA replication usually occurs at a unique site on the DNA molecule—the replication origin—by covalent attachment of deoxyribonucleotides to a short chain of primer RNA¹, designated origin RNA². A few replicons, however, have been reported to contain and sometimes utilise more than one replication origin (*Drosophila melanogaster* chromosomal DNA³; mitochondrial DNA oligomers⁴; *Escherichia coli* plasmids NR1 (ref. 5) and RSF1040 (ref. 6)), although little is known about the control of origin usage in such systems.

To gain information on how multiple regions of replication activity (replication origin plus associated essential replication functions) present on a single DNA molecule function and interact, we have constructed a model replicon (the pSC134 plasmid) by *in vitro* linkage of the colicin plasmid ColE1 and the tetracycline resistance plasmid pSC101 from *E. coli* at their unique *EcoRI* restriction endonuclease cleavage sites⁷. Since the ColE1 plasmid cannot replicate in bacteria deficient in DNA polymerase I⁸ and the pSC101 plasmid cannot replicate in bacteria not actively engaged in protein synthesis⁹, the two components of the pSC134 composite plasmid have functionally distinguishable replication properties. It was possible therefore to show that each component replication system of the hybrid could accomplish replication of the entire molecule. The present study is an investigation of the functioning of the two replication origins of the pSC134 composite plasmid and its incompatibility properties.

Location of replication origin of pSC101

Treatment of the composite plasmid pSC134 with *EcoRI* restriction endonuclease generates unit length linear forms of the parent plasmids, ColE1 and pSC101, which can be distinguished easily in the electron microscope by their different sizes (4.2 and 5.8 megadaltons respectively^{9,10}). Although it is largely possible to distinguish replication origin usage by the two components of pSC134 by determining the size of the *EcoRI* endonuclease-generated fragments which contain replication 'eyes', the unambiguous identification of such fragments

depends on the determination of origin location, since preparations of *EcoRI*-cleaved DNA may contain some fragments produced by random breakage. The replication origin of plasmid ColE1 has previously been found to lie 18% of the molecule length from the unique *EcoRI* cleavage site of this plasmid. Replication proceeds unidirectionally from this origin¹¹⁻¹³. The location of the replication origin of the pSC101 plasmid has not been reported, however, and to identify unequivocally pSC101 origin usage by the pSC134 hybrid, we first determined the location of the replication origin of the pSC101 plasmid in relation to the unique *EcoRI* cleavage site of the plasmid.

Replicating molecules of pSC101 were prepared by standard methods^{6,11} from radioactive thymidine pulse-labelled cells of *E. coli* K12 (pSC101) (Fig. 1a) and examined by electron microscopy after X-ray treatment to relax supertwisted DNA or after *EcoRI* endonuclease digestion (Fig. 1b). Circular replicating molecules were observed to contain a single replication eye, which suggested that the pSC101 plasmid probably contains a single replication origin. This interpretation was confirmed by an examination of *EcoRI* endonuclease-cleaved replicating molecules. Linear molecules which contained a replication eye were photographed (Fig. 1b) and the unreplicated arms and newly replicated segments were measured (Fig. 1c). It may be seen that for molecules less than 50% replicated, one unreplicated arm remains constant in size, whereas the other decreases progressively in length. The pSC101 plasmid therefore replicates unidirectionally. Extrapolation of the plots of relative lengths of unreplicated arms as a function of percentage replication to 0% replication indicates that the pSC101 origin is located about 50% of the unit length from the *EcoRI* cleavage site.

Replication origin usage by pSC134

To determine whether one or both of the potential replication origins of the pSC134 composite plasmid are active during normal replication of the pSC134 plasmid (that is, in conditions in which neither set of replication functions is deliberately inhibited) and to measure the relative activity of each of the possible origins, replicating molecules of pSC134 were prepared and examined as described for the pSC101 plasmid. Of 97 circular replicating molecules observed (see Fig. 2a for example), only one seemed to have two replication eyes (Fig. 2a, 6). Thus, utilisation of both origins during replication of a single molecule is very rare. Indeed such an event may be an aberrant replication process which would fail to produce functional progeny molecules.

Examination of *EcoRI* cleaved replicating forms of pSC134 revealed that the ColE1 replication origin was used exclusively during normal replication of the composite plasmid: all of 36 *EcoRI*-generated fragments of pSC134 which contained a replication eye had molecular lengths similar to that of ColE1 (Table 1). Furthermore, a plot of the relative lengths of the unreplicated arm against the extent of replication of these fragments (Fig. 2a) indicated that replication of the composite plasmid begins 18% from the nearest *EcoRI*-generated terminus of the ColE1 segment and proceeds unidirectionally away from

that terminus, as reported previously for ColE1 (refs 11–13). Thus, the location of the replication origin and the direction of replication from this origin in the pSC134 composite plasmid are identical to those of the ColE1 parent.

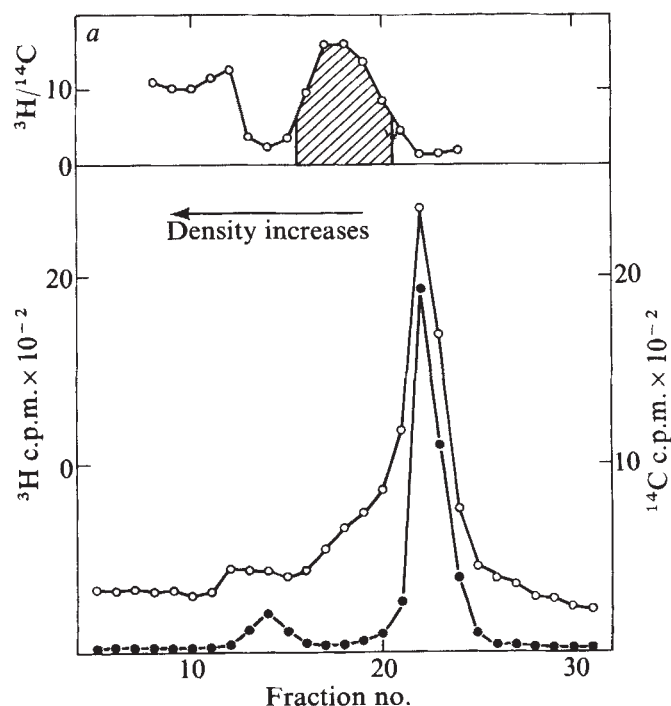
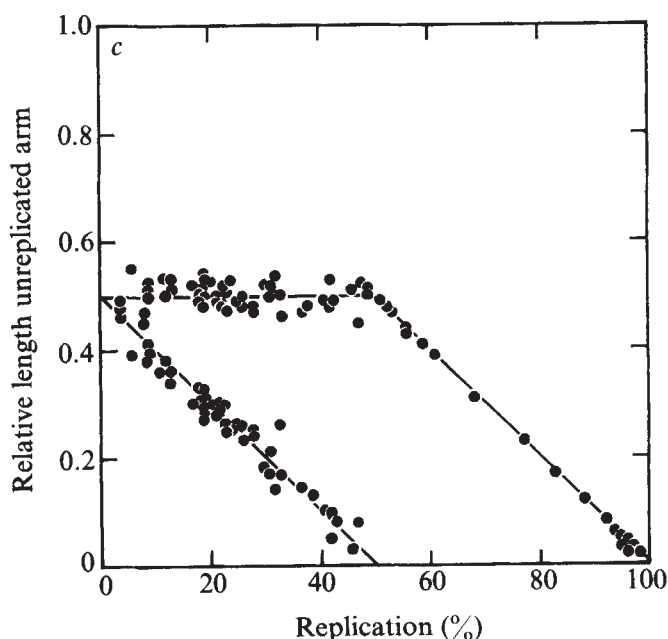
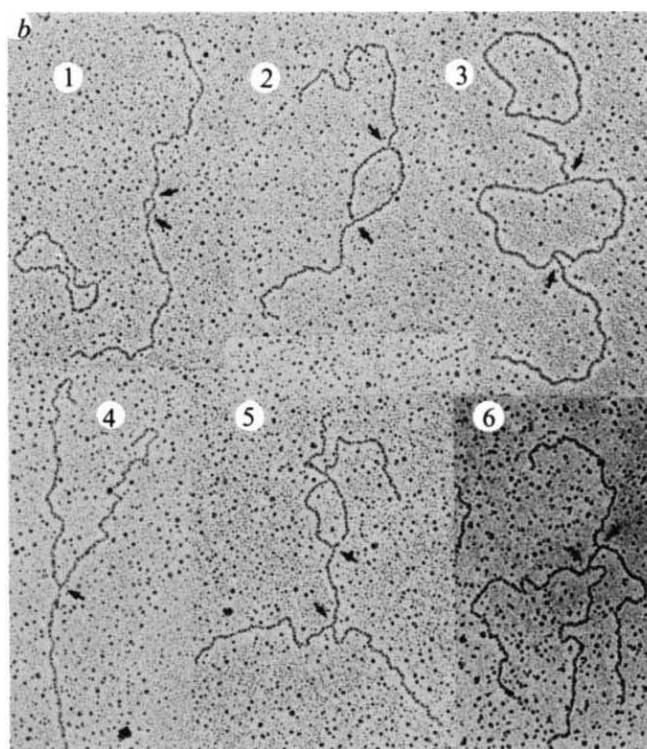


Fig. 1 Location of replication origin of plasmid pSC101. *a*, Isolation of replicative intermediates. Cells of *E. coli* K12 CR34 (ref. 34) *nal^R* (pSC101) were grown with aeration at 37 °C in 250 ml M9 minimal medium containing 0.5% casamino acids, 10 $\mu\text{g ml}^{-1}$ thiamine, 3 $\mu\text{g ml}^{-1}$ thymidine and 0.4 $\mu\text{Ci ml}^{-1}$ ^{14}C -thymidine to an A_{590} of 0.4, centrifuged at 8,000*g* for 5 min at 20 °C, and resuspended in fresh medium without thymidine. The culture was starved for thymidine by incubation at 37 °C for 30 min. It was then cooled to 20 °C and pulse-labelled by addition of thymidine to 0.3 $\mu\text{g ml}^{-1}$ and ^3H -thymidine to 10 $\mu\text{Ci ml}^{-1}$. The pulse was terminated after 30 s by pouring the culture into a large flask which was cooled in a dry ice-isopropanol bath and which contained ice and KCN (final concentration 20 mM). Cells were washed twice with ice-cold TE-KCN (Tris-HCl 10 mM pH 8.0-EDTA 1 mM-KCN 20 mM) resuspended in 2.5 ml 25% sucrose in Tris-HCl (0.25 M pH 8.0)-KCN (20 mM) and lysed by the successive addition of 0.5 ml lysozyme (10 mg ml^{-1}), 1 ml EDTA (0.25 M, pH 8.0) and 4 ml of 10% Triton X-100 in Tris-HCl (0.05 M pH 8.0)-EDTA (0.063 M). The lysate was then centrifuged at 50,000*g* for 15 min at 0 °C to remove the bulk of chromosomal DNA and the supernatant fluid was added to 8 g CsCl plus 1 ml of ethidium bromide (10 mg ml^{-1}) and was subjected to equilibrium centrifugation (at 36,000 r.p.m. for 60 h at 20 °C in the Beckman 50 Ti rotor). Fractions were collected from the bottom of the centrifuge tube and the cold trichloroacetic acid precipitable radioactivity present in a 25- μl aliquot of each fraction was determined. The lower panel shows the distribution of the ^{14}C radioactivity (uniformly-labelled DNA) and the ^3H radioactivity (pulse-labelled DNA). The upper panel shows the ratio of ^3H to ^{14}C . As has been observed for several circular DNA molecules¹, the pulse-labelled material is found predominantly between the closed circular and open circular positions in the CsCl-ethidium bromide gradient. This indicates that replicative intermediates of the pSC101 plasmid have a closed-circular duplex component. It should be noted that some pulse label is found at a more dense region of the gradient than that where the closed circular plasmid DNA bands. The pulse-labelled DNA which sediments at this position represents a novel form of replicative intermediate which has also been observed recently by others (J. H. Croso, personal communication; Y. Kupersztoch, personal communication) and which will be described in detail elsewhere (K.T., F.C., and S.N.C., in preparation; J. H. Croso, L. K. Luttrupp and S. Falkow, in preparation). *b*, Electron microscopy of *EcoRI*-cleaved replicative intermediates. The material from the CsCl-ethidium bromide gradient represented by the hatched area in *a* was extracted with isopropanol

Coexisting independent pSC101 and ColE1 plasmids cannot provide in *trans* replication functions for each other⁷, suggesting that our previously observed replication of the composite plasmid in conditions of DNA polymerase I deprivation



to remove the ethidium bromide, dialysed 3 h at 4 °C against 1 l of TE buffer (two changes), treated with *EcoRI* (ref. 35) for 15 min at 30 °C and the DNA mounted for electron microscopy using the basic protein film technique³⁶ as described by Davis *et al.*³⁷ (CoLD-CA23 (ref. 7), was used as an internal contour length reference). Grids were rotary shadowed with platinum-palladium and examined in a Philips EM201 electron microscope. *EcoRI*-cleaved molecules containing replication eyes or forks were photographed and measured and the fractional lengths of replicated and unreplicated segments were calculated and plotted as shown in *c*. Replicating molecules displaying whiskers³⁸, which were assumed to have arisen by the process of branch migration during the isolation period, were ignored for the purposes of this experiment.

occurred by means of the pSC101 origin. To test this directly, we prepared replicating molecules of pSC134 from bacteria deficient in DNA polymerase I (*E. coli* W3110 *polA1*, ref. 14) and determined replication origin usage as described above. It was found that in such bacteria, the pSC134 hybrid utilises its pSC101 replication origin exclusively (Table 1) providing direct evidence that the pSC101 replication origin can function in the hybrid plasmid.

Plasmid copy number

Although we have shown previously that both ColE1 and pSC101 replication functions are individually competent to replicate the entire pSC134 molecule⁷, it was not known whether one or both sets of functions are involved in the normal replication of the hybrid plasmid. Nordström, and Kool and Nijkamp have shown that for the R1 (ref. 15) and CloDF13 (ref. 16) plasmids, at least one product that controls the number of copies of a plasmid in a cell is encoded by the plasmid. Copy number seems to be a property of plasmid replication and may be regulated by a repressor of replication; copy number mutants frequently show altered incompatibility properties¹⁷. Moreover, the replication, copy number and incompatibility functions were shown to be clustered on a single *EcoRI*-generated DNA fragment in the case of the *F'-lac* and R6-5 plasmids¹⁸.

Since the replication functions of the pSC101 and ColE1 plasmids are apparently active only on the homologous replication origin (above, and ref. 7), exclusive use of the ColE1 origin during normal replication of the composite plasmid implies that ColE1 replication functions are being used. ColE1 and pSC101 have distinct copy numbers in logarithmically-growing cells (see below); thus determination of copy number of the composite plasmid should elucidate which of the parent plasmid copy number regulating factors is active in its replication.

Table 1 shows that under non-restrictive conditions of bacterial cell growth, the ColE1 and pSC101 plasmids have average copy numbers of 18 and 6 per genome equivalent, respectively. Coexistence of independently-replicating ColE1 and pSC101 plasmids in the same cell does not appreciably affect their relative copy number. In our experimental conditions, the pSC134 hybrid plasmid has a copy number of 16 and so exhibits the copy number characteristic of its ColE1 component. If ColE1 replication functions are inhibited by DNA polymerase I-deprivation, however, the composite replicon is maintained at a copy number typical of the pSC101 plasmid (Table 1). Thus, plasmid copy number correlates with and seems to be an indicator of the system that is being used to replicate the hybrid plasmid.

Tetracycline resistance level

The plasmid copy numbers given above were determined by direct quantitation of plasmid DNA by density gradient centrifugation; they are supported by data showing the level of tetracycline (Tc) resistance expressed by the pSC134 hybrid and its parent pSC101 plasmid. The minimum inhibitory concentration (MIC) of Tc for cells carrying the pSC101 plasmid is about 30 $\mu\text{g ml}^{-1}$ whereas the MIC for cells carrying the pSC134 plasmid is about 80 $\mu\text{g ml}^{-1}$ (Table 1). The increased resistance specified by the pSC134 plasmid is not simply due to the presence of ColE1 in the cells since bacteria carrying pSC101 and ColE1 as independent replicons are resistant to only 30 $\mu\text{g ml}^{-1}$ of antibiotic. Furthermore, the observed increased resistance is not the result of changes in transcription/translation of the Tc-resistance gene in the pSC134 plasmid as a consequence of hybrid formation, since the hybrid confers resistance to only 30 $\mu\text{g ml}^{-1}$ of Tc in cells deficient in DNA polymerase I, which maintain pSC134 at the same copy number as pSC101. We conclude that the increased resistance to Tc of cells carrying the pSC134 plasmid must result from increase in the number of Tc gene

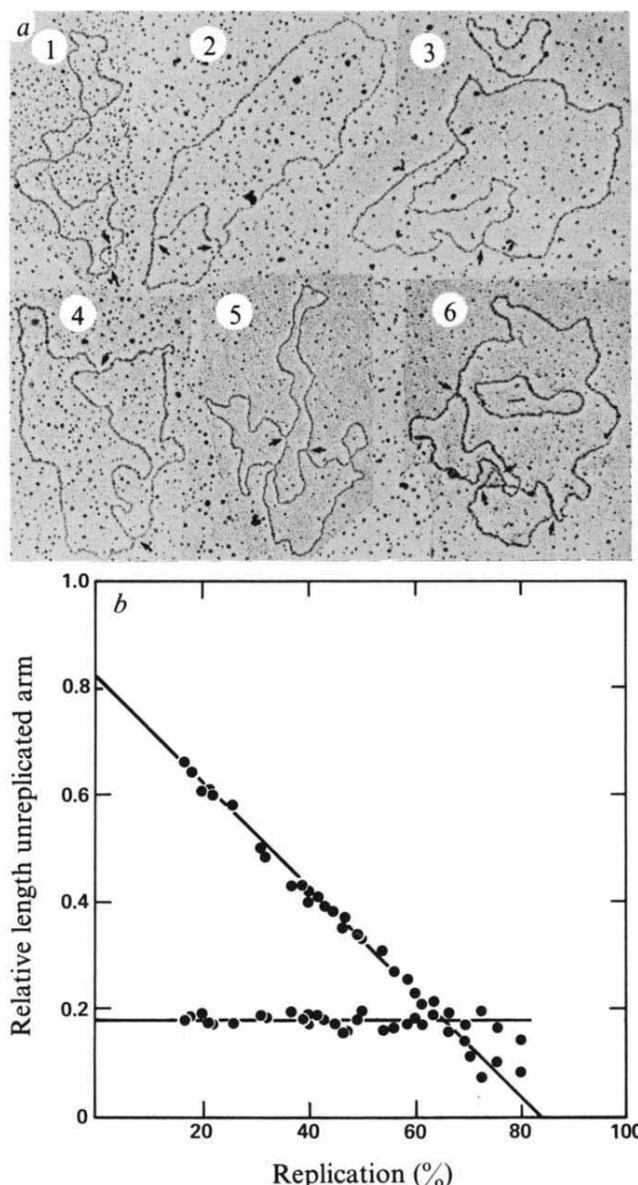


Fig. 2 Plasmid pSC134 normally utilises its ColE1 replication origin. Replicative intermediates of plasmid pSC134 were isolated as described in Fig. 1 and either nicked by X irradiation to relax supercoiled molecules (a) or treated with *EcoRI*. The molecules were then examined by electron microscopy as described in Fig. 1. The small molecules present in 3 and 6 are ColD reference molecules. *EcoRI*-generated fragments containing replication eyes or forks were photographed and measured. All had contour lengths corresponding to that of ColE1. Fractional lengths of replicated and unreplicated segments of these molecules are displayed in b. It can be seen that the replication origin on these fragments is located 18% from one *EcoRI*-generated terminus, as has been previously shown for ColE1¹¹⁻¹³.

copies (plasmid copy number) from 6 to 16 per genome equivalent and reflects a gene dosage effect.

Unlike most other kinds of plasmid-borne antibiotic resistance, in which plasmid-specified antibiotic-modifying enzymes are produced, Tc resistance seems to result from plasmid-induced changes in bacterial cell membrane permeability¹⁹. Thus there has been discussion as to whether Tc resistance could exhibit gene dosage effects²⁰, as has been observed for plasmid-specified proteins^{15, 21, 22}. Our data and the recent results of

Table 1 Replication origins and functions utilised by plasmid pSC134

<i>E. coli</i> strain	Replication origin ColE1	usage pSC101	Plasmid copy number	Level of Tc resistance
CR34 <i>polA</i> ⁺ (pSC134)	36	0	16	80
W3110 <i>polA</i> 1 ⁻ (pSC134)	0	14	6	25
CR34 <i>polA</i> ⁺ (pSC101)	—	—	5	25
C600 <i>polA</i> ⁺ (ColE1, pSC101)	—	—	18, 5	30

Origin usage by pSC134 in cells either wild type (*E. coli* CR34, ref. 34) or deficient (JG112 = W3110 *polA*1⁻, ref. 14) for DNA polymerase I was determined by electron microscopy of *Eco*RI-cleaved replicative intermediates prepared as in Fig. 1 (only a few replicating pSC134 molecules were observed in DNA prepared from strain JG112). The plasmid copy number is a minimum value of the number of plasmid copies per genome equivalent. Values obtained by centrifugation of Sarkosyl crude cell lysates to equilibrium in CsCl-ethidium bromide gradients⁷ and by sedimentation of cleared lysates through neutral sucrose gradients⁷ were in close agreement. The level of tetracycline (Tc) resistance exhibited by the different bacteria is expressed as a minimum inhibitory concentration (MIC) in $\mu\text{g ml}^{-1}$, as determined by spreading about 100 cells on nutrient agar plates containing the antibiotic at different concentrations. The copy number and Tc-resistance level characteristics of the pSC101 plasmid were comparable in *polA*1 and *polA*⁺ bacteria.

Clewell *et al.*²³ indicate that plasmid specified Tc resistance is affected by changes in gene dosage.

Incompatibility of pSC134 with parent replicons

Elucidation of the relationship between use of a particular replication system by a plasmid and the plasmid's incompatibility properties was an important objective of the present study. Earlier studies of incompatibility between related plasmids have been restricted to replicons which have acquired distinguishing phenotypic traits by genetic recombination *in vivo* (ref. 24 for example). Although incompatibility of recombinant plasmids with the parent replicons or related plasmids has been demonstrated^{42,43}, the propinquity of incompatibility properties to plasmid replication could not be determined. DNA cloning methods²⁵, however, now enable the construction of marked plasmids with identical replication systems. A series of *Eco*RI restriction endonuclease-generated DNA fragments that code for different antibiotic resistance genes (Ap, Km, SuSm) have recently been isolated from antibiotic resistance plasmids, and have been individually inserted *in vitro* into the separate ColE1 and pSC101 replicons (refs 18, 22, 25, 26 and K.T., F.C., and S.N.C., in preparation). Using such constructed molecules, the clonal inheritance of plasmids known to have identical replication functions can easily be followed during incompatibility studies by means of their different antibiotic resistances.

Incompatibility studies were carried out for the pSC134 composite replicon using ColE1-Ap, ColE1-SmSu, pSC101-Km, and pSC101-Ap derivative plasmids. Our results indicate that pSC134 is incompatible with both parent plasmids, despite its normal use of the ColE1 replication functions and origin only. The rate of segregation of pSC134 and ColE1-SmSu (Fig. 3c) is comparable with the segregation rate observed with the ColE1-Ap and ColE1-SmSu plasmids (Fig. 3d). In contrast, the segregation rate observed between pSC134 and pSC101-Km (Fig. 3a) is much greater than between pSC101-Km and pSC101-Ap (Fig. 3b).

Models for the control of initiation of DNA replication

The studies reported here have used a specifically designed and constructed composite plasmid replicon for investigation of control mechanisms regulating plasmid replication, copy number, and incompatibility. Our results indicate that although the hybrid plasmid replicon pSC134 can use either of its two component replication systems, in normal conditions only its ColE1 replication functions and origin are used. The copy number of the composite plasmid is maintained at the ColE1 level. When the ColE1 replication functions are inhibited by DNA polymerase I deprivation, control of replication occurs by means of the origin and functions of the pSC101 component, and the copy number falls to a level typical of that plasmid. Moreover, although only the ColE1 replication system is

normally used by the composite plasmid, in such circumstances it is nevertheless incompatible with both of its parent replicons. These various features of replication of the pSC134 composite plasmid must be taken into account by any suitable model for control of the initiation of DNA replication.

The replicon model of Jacob, Brenner and Cuzin²⁷, a positive control model, postulates that replication and maintenance of replicons takes place on cell membrane attachment sites. Replication occurs when the sites are duplicated and progeny molecules are subsequently distributed among daughter bacteria at cell division. According to this model, compatible plasmids have different membrane attachment sites, whereas incompatible plasmids compete for the same sites; incompatible plasmids initially present in the same cell thus segregate at cell division. Since both ColE1 and pSC101 replication origins and functions have been shown to be available for replication of the pSC134 composite plasmid, a prediction of the model of Jacob *et al.* is that each of the component systems should be used to replicate some plasmid molecules. Therefore, this model would predict that pSC134 should have a copy number similar to the additive plasmid copy number in cells carrying independently-replicating ColE1 and pSC101 plasmids, since each component replicon should be capable of producing its normal complement of copies whether or not it is physically linked to a second replicon. Finally, the model of Jacob *et al.* predicts that the pSC134 plasmid should be compatible with its pSC101 parent, since the hybrid replicon uses only its ColE1 replication origin and functions for normal replication, and thus should not compete for any pSC101-specific bacterial cell membrane replication sites. These predictions are not fulfilled by our experimental findings.

An alternative model involving negative control of initiation of DNA replication proposed by Pritchard, Barth, and Collins²⁸ postulates that a replication repressor substance is involved in the control of initiation of DNA synthesis. Replication is initiated when the repressor is diluted out to a critical concentration during the course of cell growth (that is, when a critical cell mass per replicon, the initiation mass, is achieved). If incompatible plasmids specify identical or similar initiation repressors, as required by this model, the total number of copies of the two plasmids could not exceed the usual copy number of one of them alone. Segregation of the two incompatible replicons would then occur at cell division. This model predicts that regardless of the replication functions used by the pSC134 hybrid plasmid, it should direct the synthesis of both types of initiation repressor, and thus should be incompatible with both parent plasmids (or derivatives thereof).

Implicit in the Pritchard *et al.* model is the expectation that a low copy number (high initiation mass) plasmid will be inhibited at low effective concentrations of its specific repressor, whereas greater effective concentrations of the repressor of a high copy number (low initiation mass) plasmid are required to inhibit its replication. Consequently, if two replicons such as ColE1 and pSC101 (which have different copy number) are fused, replication of the hybrid will be controlled by the ColE1 component²⁹. Replication of this component will be prevented

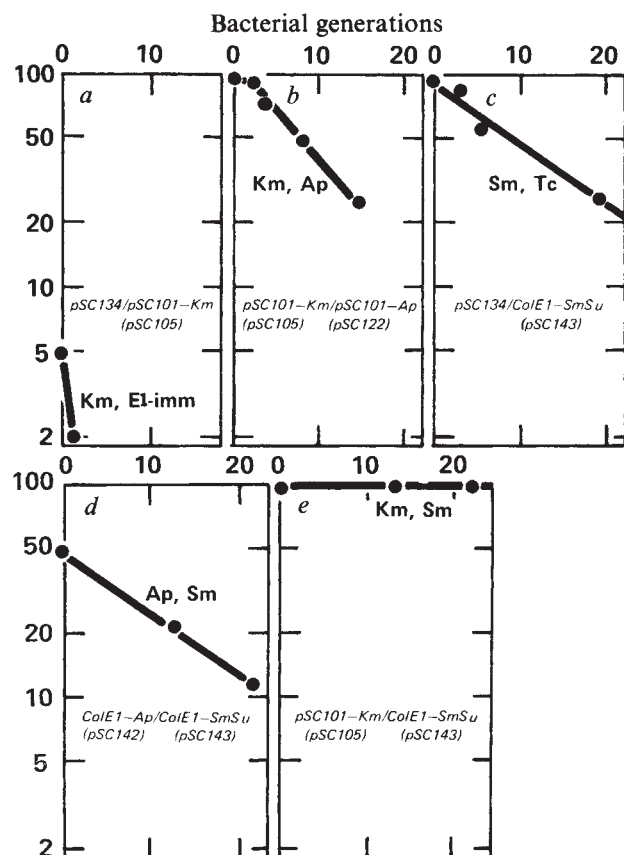


Fig. 3 Incompatibility properties of pSC134. The *in vitro* construction of the pSC101 derivatives pSC101-Ap (pSC122) and pSC101-Km (pSC105) which code for resistance to ampicillin and kanamycin, respectively, has been described^{18,25}. ColE1-Ap (pSC142) was constructed by insertion of Ap-DNA isolated from pSC122 into the *EcoRI* site of ColE1. ColE1-SmSu (pSC143) contains an *EcoRI* fragment coding for resistance to streptomycin (Sm) and sulphonamide (Su) from R6-5 (K. T., F. C., and S. N. C., in preparation). Plasmids pSC134, pSC142, and pSC105 were introduced into *E. coli* K12 HfrC (strain W2252 kindly provided by A. Ganesan) by transformation³⁹. After transformants had been purified by single colony isolation, their donor ability was determined using MS2 bacteriophage. These plasmids were then transferred by conjugation to *E. coli* K12 CR34 NaI^r bacteria containing pSC105, pSC122, or pSC143. Transconjugants were selected on media containing nalidixic acid (50 $\mu\text{g ml}^{-1}$) and the combinations of antibiotics required to select both plasmids. Antibiotics were used at the following concentrations: Ap and Km 50 $\mu\text{g ml}^{-1}$, Sm 25 $\mu\text{g ml}^{-1}$, and Tc 10 $\mu\text{g ml}^{-1}$. Transconjugant colonies which appeared on the double selection plates were used directly for segregation studies. Segregation of the plasmids was followed by picking colonies to flasks containing L-Broth plus nalidixic acid and allowing the bacteria to grow for about 25 generations in the absence of other antibiotics. At intervals samples were plated on nutrient agar. Colonies which appeared on these plates subsequently were toothpicked to nutrient agar plates containing appropriate antibiotics to determine which plasmids were present in the clone. The data plotted indicate the fraction of cells containing both plasmids. The segregation pattern thus obtained for the various plasmid combinations is shown in panels a-e. Because the pSC134 plasmid and pSC101 derivatives code for Tc resistance, the segregation of pSC134 in cells carrying pSC101 derivatives was monitored on colicin E1-containing media by means of the colicin E1-immunity (E1-imm) coded by pSC134 (colicin E1 was prepared and assayed as described in refs 40 and 41). In the panels, the first component of each plasmid pair is the incoming plasmid and the second component is the resident. With all plasmid combinations except the one represented in panel a, the incoming plasmid could be either member of the pair. The pSC105 plasmid, however, could not be introduced into bacterial cells already carrying pSC134. In a and d it can be seen that some segregation had already occurred in the clones selected before the beginning of the experiment. This is probably due to destruction of antibiotic in and around the clone by enzymes specified by one plasmid.

only when the hybrid plasmid reaches the high copy number required to produce effective inhibitory concentrations of the ColE1 replication repressor substance, whereas pSC101 replication is prevented by the pSC101 repressor substance at low copy number. Because the pSC101 repressor is always in excess when copy number of the composite plasmid is high, replication activity of the pSC101 component of the composite plasmid should be prevented so long as the ColE1 replication system is functioning. A second pSC101 replicon that is introduced into a cell carrying pSC134 (Fig. 3a) similarly should be prevented from replicating. Inhibition of the ColE1 replication system of the composite replicon results in a drop in plasmid copy number to a level where the pSC101 replication system is now able to act. When copy number of the pSC101 replicon is not forced up by its attachment to the ColE1 replicon (that is, when the two plasmids coexist autonomously in the same cell) pSC101 is able to maintain its own control of copy number and utilise its own replication origin and functions despite the presence of ColE1.

The replication properties of the pSC134 composite plasmid seem not to be explicable in terms of the positive control replicon model. The simplest interpretation of our results implicates a replication origin-specific, freely diffusible plasmid gene product that functions by negative control of DNA synthesis and, to that extent, our findings support the general model for DNA replication control proposed by Pritchard *et al.* The present studies do not, however, concern the other major aspect of this model, namely the suggestion that the timing of initiation of DNA replication is controlled by the process of dilution of the initiation repressor during cell growth. An adequate explanation for phenomena such as relaxed replication (for definition see ref. 7) of certain plasmids in non-dividing stationary phase bacterial cultures may require a more elaborate concept than that of inhibitor dilution.

Recent evidence indicates that discrete segments of DNA can be translocated from plasmid to plasmid by non-reciprocal recombinational events³⁰⁻³³. Such events could lead to the formation of naturally-occurring replicons with multiple replication origins and functions. Experimentally, such plasmids may appear to contain a single replication origin if their component segments are derived from replication systems that specify different copy number, as is pSC134. Conversely, we can speculate that replicons which are observed to use more than one origin during normal replication are derived from plasmids with similar copy number. Presumably this may occur by the recombination of genetically different replication systems which coincidentally specify a similar copy number, by recombination of closely related replication systems derived from different plasmids, or by duplication of the origin of a single plasmid.

Since the pSC134 hybrid plasmid can utilise either one of its replication systems to propagate itself in conditions that are restrictive for the other region of replication activity, it is a bifunctional replicon. Other such replicons can be constructed to contain dual replication regions derived from diverse biological sources; they are potentially highly useful for the development of DNA cloning systems for hosts other than *E. coli*. Moreover, the ability of the pSC134 plasmid to propagate in *E. coli* by using either of its replication systems now provides a valuable tool for the isolation of plasmid mutants that are defective in one of the pSC134 component replicons.

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letters to nature

Can 'invisible' bodies be observed in the Solar System?

MANY theories of the origin of comets predict that there are $\sim 10^{11}$ comets in the Solar System; most of them, unfortunately, at such a distance that they can never be observed. Some theories go even further and predict that, in addition to these comets in the Oort cloud, the remains of a primaeval comet belt may still exist at a distance of ~ 50 AU. This idea has been investigated by Hamid *et al.*¹, who show (from an analysis of observed perturbations on Halley's comet) that the total mass of the comet belt must be $\lesssim 1$ Earth mass. Even this relatively small mass would be enough for $\sim 10^{10}$ comets—a significant fraction of the total of the whole Oort cloud. It has been noted that comets contain a relatively large fraction of 'heavy' elements, and it has been suggested² that the total galactic population of comets may be large enough to affect significantly the chemical evolution of the Galaxy by acting as a 'sink' for the heavy elements.

The absence of observations of strongly hyperbolic orbits places a limit on the total mass of interstellar comets, but there remains the possibility that a great many comets may be permanently bound to their 'parent' star, and will thus always remain unobserved. Whipple³ has considered this possibility and concludes that a mass $> 0.01 M_{\odot}$ could be distributed as comets in the inner region of the Oort cloud (from 50 to $\sim 20,000$ AU) and continue to remain undetected by present methods. It is clear that techniques based on the gravitational effect of hypothetical comets on observed ones are not yet sufficiently well developed to limit speculation, and it is my purpose to suggest a way in which this might be done.

Consider a 'boulder' in the Solar System of radius R and geocentric distance d . Its angular diameter is $\theta = 2R/d$; similarly, the angular diameter of a star is $\theta_* = 2R_*/d_*$. The boulder can occult the star if $\theta > \theta_*$, that is if

$$Rd_*/R_*d > 1 \quad (1)$$

If the geocentric transverse velocity of the boulder is v_t it will seem to move its own diameter in a time, τ , where $\tau = 2R/v_t$.

The boulder therefore 'sweeps' a narrow track on the celestial sphere at a rate

$$\dot{A} = \theta^2/\tau = 2Rv_t/d^2 \quad (2)$$

Should there be n such boulders per steradian the whole sky can be considered to have been swept after a time T , where $nAT \simeq 1$, giving

$$T \simeq d^2/2nRv_t \quad (3)$$

If one is able to monitor n_* stars continuously, each satisfying the criterion (1), on the average one should observe an occultation once every

$$T' \simeq d^2/2nn_*Rv_t \quad (4)$$

As an example, we shall consider the possibility of detecting the hypothetical comet belt at ~ 50 AU. To obtain a rough estimate of T' we shall suppose that the belt consists of N identical comet nuclei, each of radius R and density ρ . If the upper limit to the mass of the belt is M_u we have

$$N \lesssim M_u/(4/3)\pi\rho R^3 \quad (5)$$

One would expect the comets to show some concentration towards the ecliptic, and as a rough approximation we shall assume that the N comets are distributed over 2 sr of the celestial sphere. This means that $n \simeq N/2$, or

$$n \lesssim \frac{1}{2} M_u/(4/3)\pi\rho R^3 \quad (6)$$

then, from equation (4)

$$T' \gtrsim 4\rho R^3 d^2/n_* M_u v_t \quad (7)$$

For $v_t = 10 \text{ km s}^{-1}$, $R = 3 \text{ km}$, $d = 50 \text{ AU}$, $M_u = 5 \times 10^{24} \text{ kg}$, and $\rho = 10^3 \text{ kg m}^{-3}$, we find

$$T' \gtrsim 4 \times 10^7/n_* \text{ s} \quad (8)$$

It is of interest to estimate the number of stars, n_* , that could be successfully monitored using existing technology. Perhaps the most suitable detector for the purpose is a SIT Vidicon

system, which scans a large area with high sensitivity and good time resolution. Noise in the system arises primarily from photon statistics, and for the following estimate of n_* it will be assumed that this is the only source of noise. Assuming that a 0 mag star produces $\sim 1,000$ photons $\text{\AA}^{-1} \text{cm}^{-2} \text{s}^{-1}$, and that the photocathode has a bandwidth of $3,000 \text{\AA}$ with a mean quantum efficiency of 15% (for example S-20), it can be shown that the contribution to the signal in a 1 m telescope from a 16 mag star is ~ 140 photon events per 0.1 s. Taking the sky background to be equivalent to one 21 mag* per square second of arc, and the resolution of the vidicon to be $10''$, we have $S_* \approx S_B$. The total signal therefore consists of ~ 280 photon events per 0.1-s interval, of which ~ 140 on average come from the star. A fairly safe criterion for the occurrence of an occultation would be to count an 'event' each time the signal in any 0.1 s interval falls below that corresponding to 160 photon events. (Assuming that the photon events obey a Poisson distribution, the probability of such a fluctuation would be $< 10^{-13}$; whereas equation (8) shows that the probability of an occultation is $\sim 2.5 \times 10^{-9}$ per 0.1 s.) The statistics could be greatly improved for the case of occultations lasting longer than 0.2 s. A vidicon tube consisting of a 300×300 array of resolution elements, each with the stated resolution, will be able to monitor ~ 0.7 square degrees of sky which, considering stars brighter than 16 mag will contain $\sim 1,000$ stars. With $n_* = 1,000$, the waiting time between occultations reduces to ~ 11 h. One consequence of the use of such a technique would be that M_u would continually decrease if no occultation was seen. It should be emphasised that the figure of 11 h observing time per 'event' could almost certainly be greatly improved if an instrument was purpose built for the detection of occultations.

The above is meant only to illustrate the possibilities for such an observational technique. A complete theory would have to be statistical in nature, and the interpretation of a particular event would depend on the distributions of R , d , and v_i that one might expect to encounter. One would like, for example, to be able to distinguish between a boulder at 1 AU and a comet nucleus at 100 AU. If the detector system could be made sufficiently sensitive to be able to record 'partial' occultations, the detection of a variety of interplanetary debris may be possible and, in particular, the detection of comets at distances $\gg 50$ AU would be possible. The technological problems involved in the design and construction of such a detector promise to be large, but should not be insurmountable. In view of the many important results likely to come from such a programme—benefitting not only cometary astronomy—it is to be hoped that someone will investigate the further possibilities of 'occultation' astronomy.

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Third discontinuity in the Vela pulsar period

THE period of the Vela pulsar, PSR0833-45, decreased discontinuously by approximately two parts in 10^6 near the beginning of October 1975, the third such event observed for this pulsar. The first^{1,2} occurred in March 1969, and the second³ in August 1971.

Since May 1975 pulse arrival-time data have been recorded for a number of pulsars at the NASA Deep Space Station,

Tidbinbilla, located near Canberra, Australia, with an average spacing between observations of ~ 10 d. A 26-m diameter antenna is being used at an observing frequency of 2,295 MHz. For the Vela pulsar 25 integrated pulse profiles are recorded on magnetic tape during an observation lasting ~ 20 min. Typically, data from such an observation give a mean arrival-time having an uncertainty of 20 μ s. Propagation times to the Solar System barycentre are computed using an ephemeris provided by I. I. Shapiro of the Massachusetts Institute of Technology. As data have not been recorded for a full year, the pulsar position cannot yet be computed from the arrival-time data; the Molonglo position, RA 08 h 33 min 39.44 s, dec $-45^\circ 00' 10.2''$ (ref. 4), was assumed.

Data recorded between 21 May 1975 and 25 September 1975 can be fitted quite closely by a parabolic curve; the parameters obtained from this fit are given as the pre-jump data in Table 1, where the errors quoted are dominated by the uncertainty in pulsar position. Significant residuals from the fitted curve, apparently resulting from intrinsic variations in the pulsar period, are observed. The r.m.s. residual for the 127-d period including 13 independent observations, is 165 μ s, large compared to the 20 μ s observational uncertainty. This pre-jump period agrees within a few parts in 10^7 with that extrapolated from parameters determined after the August 1971 event³, implying that no similar jump occurred between then and May 1975.

Pulse arrival times obtained on and after 15 October do not lie on the parabola extrapolated from these previous data. A least-squares fit of a parabola to data recorded from 15 October to 14 November gives the post-jump parameters listed in Table 1. Based on extrapolation of the pre-jump data, the period at JD 2442700.5069 would have been $0.089\,234\,889\,53 \pm 15$ s. The magnitude of the period change was therefore 175.6 ± 0.2 ns, corresponding to $|\Delta P|/P = \Delta\Omega/\Omega = 1.97 \times 10^{-6}$, and to a phase drift of about 1.9 cycles d^{-1} with respect to the pre-jump period. Because of this large drift it is not possible (by extrapolating the post-jump data backwards) to determine on which day the period decrease occurred. It can only be said that it occurred between JD 2442680.64 (September 25) and JD 2442700.51 (October 15).

Table 1 Timing parameters for the Vela pulsar

Pre-jump data	(JD 2442553-2442680)
Period	$0.089\,233\,318\,26 \pm 7$ s (A.1)
dP/dt	$(124.096 \pm 0.010) \times 10^{-15} \text{ s s}^{-1}$
Epoch (JD)	2442553.9595
Post-jump data	(JD 2442700-2442731)
Period	$0.089\,234\,713\,88 \pm 7$ s (A.1)
dP/dt	$(125.03 \pm 0.01) \times 10^{-15} \text{ s s}^{-1}$
Epoch (JD)	2442700.5069

The data in Table 1 also show that there was an increase in the rate of change of the period of $(0.93 \pm 0.02) \times 10^{-15} \text{ s s}^{-1}$, apparently coinciding with the decrease in the period. The corresponding value of $\Delta\dot{P}/\dot{P} = |\Delta\dot{\Omega}|/\dot{\Omega}$ is 7.5×10^{-3} . Because of possible systematic variations in the period and its derivatives following the jump, and the superimposed random irregularities, the post-jump parameters must be considered preliminary values only.

The parameters of this third jump are remarkably similar to those of the previous jumps in spite of the fact that the interval between the second and third jumps is over 60% greater than that between the first and second. Values of the period decrease for the 1969, 1971 and 1975 events are, respectively, ~ 200 ns, 178.6 ns and 175.6 ns. For the 1969 event the period derivative increased by $\sim 1.00 \times 10^{-15} \text{ s s}^{-1}$, compared to $0.93 \times 10^{-15} \text{ s s}^{-1}$ for the 1975 event. The increase in dP/dt for the 1971 event was comparable.

Further analysis of the variation in period for before and after this third discontinuity will be undertaken when additional post-jump data are available.

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B-emission stars and X-ray sources

SEVERAL transient X-ray sources with decay times of the order of weeks to months have been reported¹; the discovery of four new sources of this type, A1118-61 (refs 2 and 3), A0535+26 (refs 4-6), A1742-28 (P. W. Sanford, personal communication), A1523-61 (ref. 7), in less than 1 yr of operation of the X-ray satellite Ariel V, indicates that these objects are rather common. Important for the understanding of this phenomenon is the optical identification of the sources: it has been proposed that, A1118-61 and A0535+26, coincide with bright B stars with emission lines ('Be' stars)^{8,9}, while A0621-00 is identified with a completely different object, which showed a nova-like behaviour optically, simultaneous with the X-ray outburst¹⁰. Here we discuss X-ray emission from systems containing Be stars.

Be stars are appealing candidates for X-ray transients for the following reasons: first, a number of these stars are single-line spectroscopic binaries with periods of ~ 10 d (ref. 11). It is suggested that many Be stars have an evolved, invisible, close companion. The very rapid rotation of these stars is then naturally explained by mass exchange from the envelope of the presently invisible (though initially more massive) companion. Second, evolutionary considerations¹² show that if some mass loss from the system occurs during the first stage of mass transfer, compact stars (neutron stars or black holes) can be formed as companions of main-sequence stars of masses as low as $8-10 M_{\odot}$. This corresponds to main-sequence spectral type as late as B3-B4 (Be stars are indeed main-sequence stars, and are generally found in the entire B-type spectral region). The lifetime of main-sequence stars of $8-10 M_{\odot}$ is $\sim 2 \times 10^7$ yr. Third, Be stars characteristically show a central absorption component in the Balmer lines, indicating the presence of circumstellar material. It is generally thought that this matter is ejected from the equatorial regions where, because of the extremely rapid rotation of the Be stars, the effective gravity is almost zero. The emission features vary over time scales of 1-100 yr (ref. 11).

We propose that sudden variations in the rate of mass ejection from Be stars in the presence of a compact companion could produce transient X-ray emission, which might recur over a period of years, like the observed optical emission of Be stars. The companion star should be a neutron star, rather than a white dwarf, because the spectrum of the transients is rather hard ($\alpha = 1.0$ for pulsating sources, and 2-4 for the others) and the luminosity rather high ($\simeq 10^{37}$ erg s⁻¹). The total amount of accreted matter required for an outburst is, for a neutron star, $\Delta M \simeq 10^{23}$ g, which is only a few per cent of the amount of mass in the envelopes of Be stars required for producing the observed emission lines.

Two of the transients (A1118-61 and A0535+26) vary periodically with very stable periods of 405 s and 104 s, respectively. If these are rotation periods for neutron stars they

are very slow, but nevertheless conceivable, since the lifetime of these systems can be $> 10^7$ yr. On such a time scale the rotation could have been slowed down by the propeller mechanism¹³. The X-ray binary Vela X-1 is a slow pulsar ($P = 283$ s (ref. 14)), and this supports the idea that these slowly pulsing transients might be neutron stars in binaries with orbital periods of the order of days. (Early B stars are observed¹⁵ to have weak winds that can provide sufficient braking for a neutron star companion on this time scale¹⁶.)

Other systems identified with objects optically similar to Be stars are 3U0352+30 (X Per) and Cen X-3 (Krziminski's star). X Per has been classified as an extremely rapidly rotating (peculiar) B0Ve star¹⁷ and Krziminski's star is an O6V-III emission star (rather close to the main-sequence, and not a clear Of star, since strong 4640 Å and 4686 Å emission is absent¹⁸). In two other cases, 3U1145-61, 3U1223-62 there are Be stars in the error boxes (HD 102567, Wray 977). If the identification with X Per is correct, 3U0352+30 has an X-ray luminosity of $\sim 10^{33}$ erg s⁻¹, which seems to be modulated with a period of 22 h (N. E. White, P. W. Sanford and K. O. Mason, unpublished). It is a remarkable fact that the maximum (break-up) rotational velocity for a B0V star corresponds to a rotation period of ~ 1 d. We therefore suggest that the X-ray period represents the orbital period of a binary system, and that X Per rotates synchronously with this orbital period. The low X-ray luminosity would arise from either a low rate of mass transfer or a source which is almost completely buried by a supercritical mass accretion rate¹⁹. Such weak sources are measurable at the Uhuru sensitivity, only to within 0.5-1 kpc. It is, therefore, possible that 3U352+30 is a 'low' state for a transient X-ray source, and is observable only because it is close (~ 170 pc).

It seems important that the X-ray behaviour of Cen X-3 is in many respects similar to that of the pulsating transient X-ray sources, in that it turns 'off' for as much as a month at irregular intervals; when it turns on, its X-ray spectrum shows intense low-energy absorption²⁰, very similar to the peak behaviour of the transients seen by Ariel V. A further similarity with these two transients is that Cen X-3 shows regular X-ray pulsations (though with a much shorter period (~ 5 s)).

If our suggestion that a class of X-ray transients is associated with Be stars is correct, it follows that the total number of these types of transients in a quiescent state is given by the number of Be transients per year (~ 4) divided by the frequency of active periods in Be stars ($\sim 10^{-1}$ yr⁻¹). Therefore—on the binary model—Be transients in a quiescent state should be approximately as numerous as steady galactic X-ray sources.

Note added in proof: After submission of this paper, 3U0352+30 (X Per) and 3U1223-62 (Wray 977) were discovered to be slow pulsars with periods of 13.924 and 11.64 min, respectively^{21,22}. This seems a strong confirmation of our above suggestion (based on their association with Be Stars) that these sources are related to the slowly pulsating transients and to sources like Cen X-3.

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Does Mercury have a molten core?

THE Mariner 10 mission has discovered a magnetic field associated with the planet Mercury¹. The preferred explanation of the source of this field based on these observations, is an internal dynamo². A necessary condition for such a dynamo is the existence of an electrically conducting liquid region within the planet. In this letter, we investigate whether or not Mercury could contain a molten metallic core.

Internal temperature distributions for a solid, undifferentiated Mercury have previously been calculated by Majeve³. Siegfried and Solomon⁴ considered the effects of melting and fractionation. They concluded that Mercury would have a metallic core fully differentiated from the silicate mantle if the uranium and thorium abundances are taken from the cosmochemical model of Lewis⁵. According to Siegfried and Solomon, such models predict a present-day temperature profile rising from the surface temperature to the melting curve in the upper 300 km, a molten mantle and a slowly cooling core either at or below the melting curve of iron. It has been pointed out^{6,7} that such a thermal constitution would prevent dynamo generation. We have extended the work of Reynolds *et al.*⁸ and Fricker *et al.*⁹ to study the possible thermal evolution of a metallic core in Mercury. The calculations involve the solution of the equation of heat conduction for a spherically symmetric body with internal heat sources. Modifications have been incorporated to take into account the latent heat of fusion, and the redistribution of radioactive heat sources as a consequence of melting. The numerical methods used follow the method described in ref. 8. For the internal structure of Mercury, the work of Reynolds and Summers¹⁰ was used. Following Lewis⁵, it was assumed that the condensation of U, Th and Fe was complete at the orbit of Mercury and that, like other volatile and chalcophile elements, K can be neglected. For a model of Mercury with a metal weight-fraction of 66%, an average U concentration of $2.25 \times 10^{-8} \text{ g g}^{-1}$ as estimated by Wasserburg *et al.*¹¹ for the Earth would correspond to a U content of $4.4 \times 10^{-8} \text{ g g}^{-1}$, if the Fe/U ratio of the Earth is applied. This value together with a Th/U ratio of 3.7 has been used. The calculated heat conduction for the silicate material relies on the data given by Schatz and Simmons¹² for the mantle material of the Earth; it includes the contributions of lattice conductivity and radiative transfer. The conductivity of iron was taken as $2.5 \times 10^6 \text{ erg cm}^{-1} \text{ K}^{-1} \text{ s}^{-1}$. The heat capacity of the silicate material was assumed to be $1.2 \times 10^7 \text{ erg g}^{-1} \text{ K}^{-1}$, that of iron $0.6 \times 10^7 \text{ erg g}^{-1} \text{ K}^{-1}$. For the bulk melting temperature of the silicates, the melting curve of diopside (Fig. 1) was adopted¹³. The surface temperature was held constant at a value of 75 °C (ref. 14).

Interpretation of the recent Mariner results suggests that extensive igneous activity has occurred on the surface

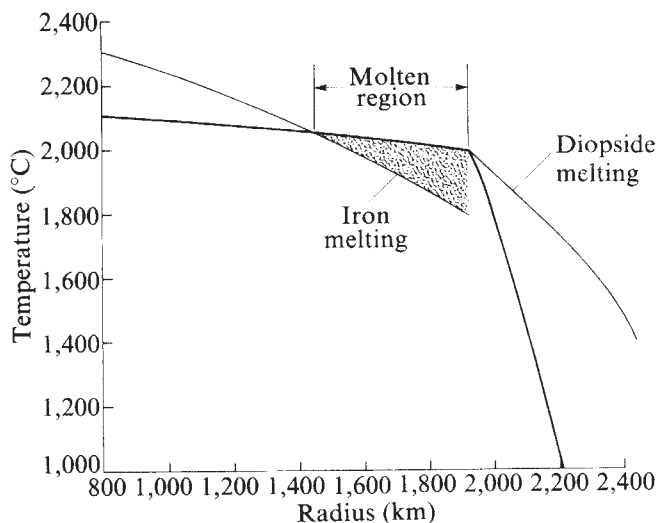


Fig. 1 The heavy curve is the calculated temperature profile of Mercury 4.6×10^9 yr after core formation. The diopside and iron melting curves are shown. The molten core region is ~ 500 km thick.

of Mercury: there is evidence that global differentiation occurred very early in the planet's history, resulting in the formation of a metallic core^{15,16}. The high initial temperatures required for an early differentiation process have evidently also prevailed in the other terrestrial planets and the moon, as well as in the relatively small meteorite parent bodies^{9,17,18}. Therefore, the calculation was started with an initial temperature distribution corresponding to the melting curve of iron^{19,20}, and the core was assumed to have formed essentially coincident with planetary formation, 4.6×10^9 yr ago. As a result of the melting and differentiation process, the radioactive heat sources are assumed to be concentrated in the outermost layers. Other assumptions regarding the initial state of the planet tend to result in higher interior temperatures at the present time, since core formation is delayed and less time is available for cooling.

The boundary between the iron core and the silicate mantle at a pressure of $7 \times 10^9 \text{ Pa}$ ($1 \text{ Pa} = 10^{-5} \text{ bar}$) coincides with a substantial difference in melting temperatures: the melting curve of diopside is ~ 200 °C higher than the melting curve of iron at this point. This difference holds also for other silicate materials, which are depleted in volatile and chalcophile elements²¹. In addition, the presence of Ni, or of other minor constituents, in the core, would tend to lower the assumed iron melting curve. The core-mantle boundary separates two contrasting thermal conductivity regimes. The differences in melting temperatures and transport properties has a crucial role in the thermal evolution of the interior of the planet. Because of the higher melting temperature in the deep mantle and the lower conductivity of the mantle material in comparison with iron, the core-mantle boundary represents an effective heat transfer barrier in the absence of efficient solid convection in the mantle. This barrier permits temperatures in the outer core to rise above the melting curve of iron, with a corresponding reduction of the temperature gradient at the core-mantle boundary. If the barrier did not exist, the heat flux from the interior would be at least equal to the product of the iron thermal conductivity and the melting curve gradient, a value sufficient to solidify the entire core in $< 2 \times 10^9$ yr. The models of Siegfried and Solomon⁴ neglect the melting temperature discontinuity, and therefore predict solidification of the core and a large molten region in the mantle.

The temperature profile predicted by the thermal history calculations for 4.6×10^9 yr, using the above initial conditions and parameter values, is given in Fig. 1. For our model, the inner 1,400 km of the core would now be solid, while the outer 500 km of the core remains molten. This model, therefore, fulfils a necessary requirement for a dynamo: at least a portion of the core is molten. It is also consistent with the apparent lack of recent degradation of surface features by magmatic activity, since the mantle is solid.

We wish to emphasise the following: the result that a molten shell exists now, and is a direct consequence of the discontinuity in melting temperatures at the core-mantle boundary. This result is independent of the density of planetary heat sources and the particular initial conditions imposed, as long as a molten core once existed and in so far as solid convection in the mantle can be ignored (see below). Core formation later than 4.6×10^9 yr ago, retention of radioactive heat sources in the core or deep mantle, and the action of other energy sources (such as tidal dissipation or electrical heating) would all result in a larger present-day molten region than in our model.

The calculations described here do not include the effects of solid convection in the mantle, which could solidify the core under certain circumstances. Cassen *et al.*²² have discussed this aspect of the problem, and find that it is necessary that a density of heat sources at least comparable with the Earth's mantle-wide average be retained throughout Mercury's mantle if the core is to remain molten in the presence of solid convection. (This requirement is additional to the melting temperature discontinuity at the core-mantle boundary.)

Finally, we note that our calculated temperature gradients in the molten region of the core are subadiabatic. Therefore, although a dynamo is possible in this model, it would have to be driven mechanically, rather than by thermal convection. Gubbins²³ has discussed the energy requirements of a convective dynamo.

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Mid-latitude ionospheric scintillation fading of microwave signals

MEASUREMENTS of the amplitude of microwave (1,550 MHz) signals from the NASA ATS-5 geostationary spacecraft were made from December 1973 to July 1974. These data have been analysed for diurnal, geomagnetic and seasonal variations, and also for dependence on the boundary of the high latitude scintillation region in the direction of the Equator.

The signal to the spacecraft, at a frequency of 1,650 MHz, was transmitted from the DOT/TSC/Westford Propagation Facility at Westford, Massachusetts (42.6°N; 71.5°W, geographic). It was produced as follows: a 2-W signal from an L-band exciter was used to drive a 200-W travelling wave amplifier. The signal was carried through 15.2 m of rigid feedline to a 4.6-m diameter parabolic reflector antenna whose feed was such that right-hand circular polarisation was transmitted. A high power narrow-band filter after the output of the final amplifier insured that no out-of-band spurious emissions were transmitted.

This signal was frequency shifted by the L-band transponder of the spacecraft, in the narrow-band frequency translation mode, to a frequency of 1,550 MHz and re-emitted. The power level in the uplink was high enough to saturate the ATS-5 L-band transponder by > 7 dB thus assuring that the amplitude variations on the downlink were 'safe' from uplink fading contamination by 7 dB.

The signal beam crossed the 350-km level in the ionosphere at 30.1°N, 75.8°W (near Philadelphia) because the ATS-5 had a subsatellite longitude of 105°W. The invariant latitude Λ was 54°N (ref. 1). ($\Lambda = \cos^{-1}(1/L)$, where L is McIlwain's coordinate².)

The downlink signal was then received at the Westford facility on three independent receiving systems where its amplitude was recorded in both analogue and digital form.

Each system consisted of a 3.1-m diameter parabolic reflector antenna with a preamplifier mounted at its spiral feed so chosen that right-hand circular polarisation was received.

Two of the receiving systems converted to a frequency of 30 MHz and then to 10 MHz, the signal from the other receiving system was converted to 136 MHz, then to 55 MHz and finally to 10 MHz. These 10-MHz signals were each filtered with a 30-kHz crystal filter, re-amplified and square-law detected. A portion of each filtered 10-MHz signal was also fed to three vector voltmeters which were used as narrow-band receivers with their 10-MHz reference signal coming from a frequency standard at the station. The vector voltmeters had a bandwidth of 1 kHz and employed a square-law detector following the narrow-band filter.

The 30-kHz detector voltage from each of the three receivers was recorded on an analogue strip-chart simultaneously with the detector voltage from each of the three vector voltmeters on a 6-channel strip-chart recorder. The calibration of the recorder was simplified by the identical calibration of the three vector voltmeters and of the three 30-kHz detected voltages.

Three independent receivers, each with a narrow- and wide-band mode, were used on the received signal to assure that the drift caused by gain instabilities could be isolated from shifts of frequency in the spacecraft's transmitter. During the measurement programme, a frequency drift in the spacecraft's transmitter was never observed.

The three detected voltages were digitised, preprocessed and recorded on magnetic tape for later computer processing³. An example of the analog data and the computer calculated statistics for April 1, 1974 are presented in Fig. 1. The strip-charts of the 30-kHz detectors are shown.

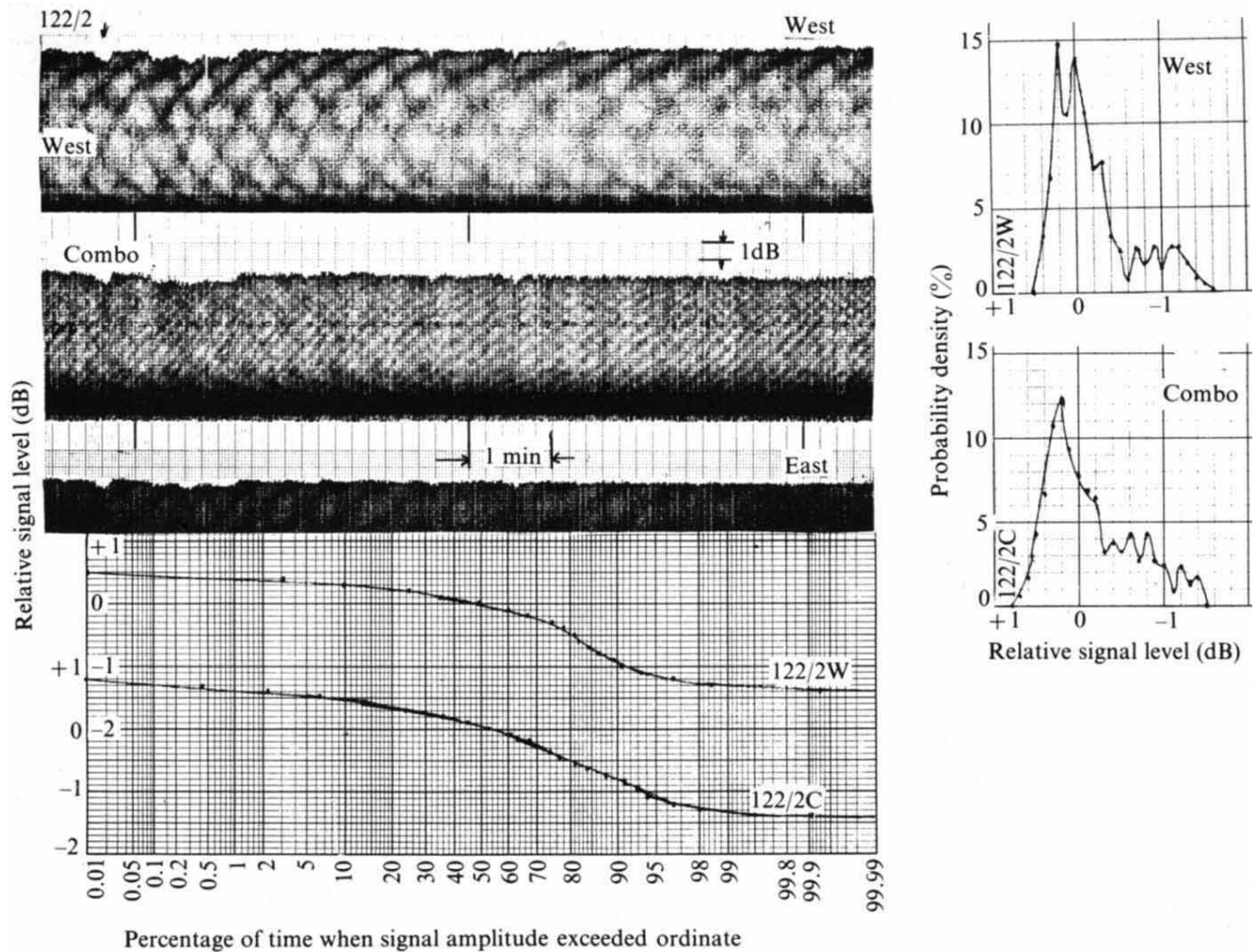


Fig. 1 Example of analog data and computer calculated statistics for scintillation on April 1, 1974. ATS-5 subsatellite longitude: 105°W ; Westford elevation angle: 30.5° ; Azimuth angle: 224°SW . The reason for the spiked fence appearance of the strip-chart recording is that the ATS-5 spacecraft was spinning with a period of 785 ms and thus traced out its L-band antenna pattern during each sweep. DOT/TSC/Westford propagation facility: NASA/ATS-5 spacecraft in NBFT Mode; WPF uplink at 1651.270 MHz; Downlink at 1550.250 MHz at $S/N = 30$ dB; BW = 1 kHz; median signal level = -117.5 dBm (West) and -120.0 dBm (Combo); Start time = 1415 EDT; file duration = 12 min.

Note how all three channels fade simultaneously. The calculated probability densities⁴ for two of the three channels are also presented, as well as the cumulative amplitude distribution for the 12-min measurement interval.

In each of the 2,500 12-min data samples the best channel (that with amplitude drift < 0.2 dB) was analysed. The 12-min data samples were statistically analysed to determine mean values, root-mean-squares (r.m.s.), probability density functions and probability distribution functions. The value of the 12-min distribution function at the 90% level was termed a 'scintillation index'. These 12-min 'scintillation index' values were grouped into 1-h and 3-h intervals to form '1-h scintillation index' (1-h SI) and '3-h scintillation index' (3-h SI) values, respectively.

The 3-h SI values were associated with the corresponding planetary magnetic index values which are computed for standard 3-h intervals⁵. The results, presented in Fig. 2 show that the higher values of magnetic activity are associated with the higher values of the 3-h SI.

Figure 3 presents the diurnal variations of the microwave ionospheric scintillation as expressed by the 1-h SI. For low Kp ($Kp < 4$) there seem to be slight maxima at 1800, 2000 and 0000 LMT. For high values of Kp ($Kp > 4$) there are slight maxima at 1800 and 0000 LMT.

Also plotted on Fig. 3 is the latitudinal variation of the mid-latitude scintillation boundary, as determined by

Kersley *et al.*⁶. The appropriate microwave scintillation data ($Kp < 4$) do not seem to be directly related to the position of the scintillation boundary as determined by the measurements at 40 MHz. There is, however, a well-defined peak during the period when the statistically averaged scintillation boundary crosses the 54° parallel. Although the two sets of data were taken at different phases of the sunspot cycle and different geographical longitudes, the data do fit together.

During the solstices the data looked relatively calm with only a few r.m.s. signal level fluctuation measurements > 0.4 dB during December and January, or June and July. During the vernal equinox, however, the r.m.s. signal level did have values in the 0.4 – 0.6 dB range as would be indicated by the conclusions of Oksman and Taurianien⁷.

The following are our conclusions so far: first, ionospheric scintillation at microwave frequencies does exist at mid-latitudes. Second, of the 250 h of observations analysed, 10.2% were found to be undisturbed with a r.m.s. fluctuation level of ≤ 0.22 dB. Also, 0.84% of the observation periods were found to have disturbed propagation conditions with the 90% levels exceeding 1 dB which corresponds to a ~ 2 dB peak-to-peak fluctuation level. Third, the largest peak-to-peak fluctuation that was observed on the strip-chart recording was 3 dB. Likewise, the smallest r.m.s. fluctuation level for an observing period was 0.15 dB.

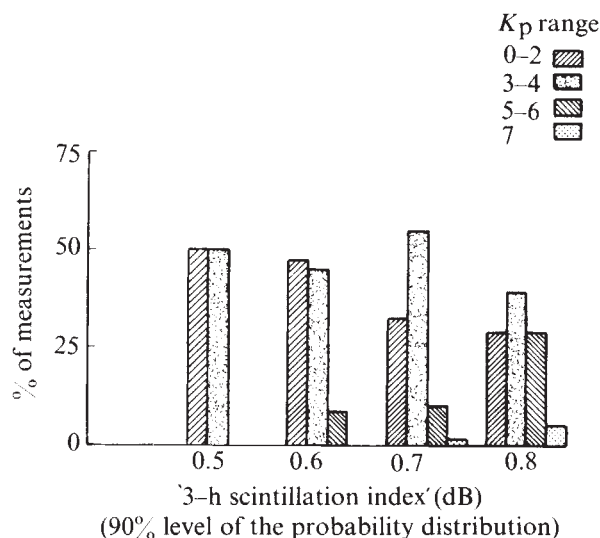


Fig. 2 Comparison of 3-h SI with the planetary magnetic index, K_p , (December 1973–July 1974).

Fourth, mid-latitude ionospheric scintillation is related to magnetic activity, in that higher levels of magnetic activity are associated with higher levels of microwave scintillation. Fifth, mid-latitude scintillation has some diurnal variations⁸. Sixth, some seasonal variation was indicated, with the smallest scintillation activity during the winter solstice, and the maximum scintillation around the vernal equinox.

Fading of the amplitude of microwave signals has been reported for equatorial regions^{9–11} and for auroral or polar

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Long conductors as antennae for gravitational radiation

To date, the antennae used in the search for gravitational radiation have been mechanical in nature, that is they involve the detection of small displacements or strains in mechanical systems. Here we demonstrate that a conceptually simple system of two long conducting wires of different metallic composition constitutes, in principle, a non-mechanical antenna for gravitational radiation, and that if the conductors are sufficiently long such a system may be of practical use. Very briefly, the principle of operation of the system is that gravitational radiation produces a greater force on the ions in a metal than on the electrons, which, to preserve charge neutrality, necessitates a compensating electric field which is, in principle, detectable.

The effect of a constant gravitational field on a conductor has been studied by a number of authors; we note in particular the theoretical work of Barnhill and Schiff^{1,2}, and of Dessler, Michel, Rorschach, and Trammell³. The result of this work is the prediction that a vertical electric field will exist inside and in the vicinity of such a conductor with magnitude

$$E = \Lambda mg/e \quad (1)$$

Here m is the mass of an ion in the metal, e is the charge of the electron, g is the acceleration due to gravity, and Λ is a constant representative of the metal, typically ~ 0.1 – 1 (ref. 3). We may understand this result qualitatively as follows: the gravitational force on the ions is mg ; on the much lighter electrons it is thousands of times less. To preserve charge neutrality it is necessary that the electrons experience an electric force eE of the same order of magnitude as mg . Equation (1) expresses the equality of these forces.

If we consider a conductor in rapid rotation it is evident that similar considerations will apply if we replace the gravitational force mg by the centrifugal force, which can be made quite large by the use of high rotation rates.

$$mg \rightarrow m\omega^2 l; E = \Lambda m\omega^2 l/e \quad (2)$$

Here ω is the rate of rotation of the conductor and l is the distance from the axis of rotation. The field predicted in equation (2) has in fact been observed, with the correct order of magnitude, in an experiment by Beams⁴. This experimental

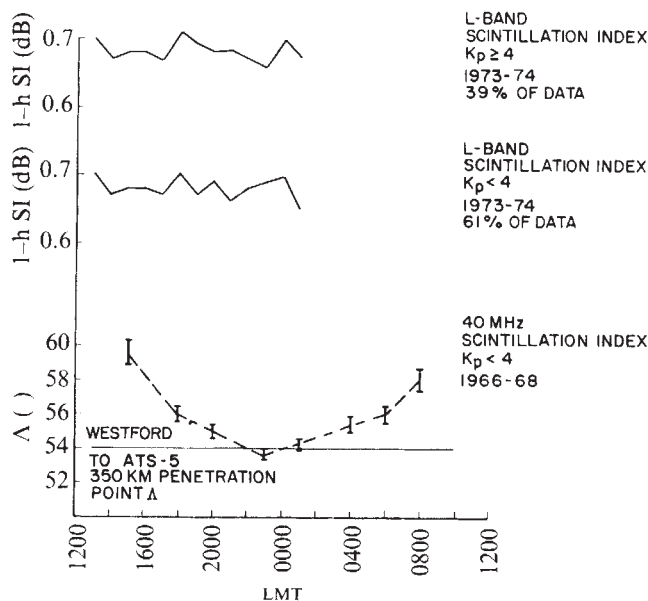


Fig. 3 Diurnal variation of microwave ionospheric scintillation for two ranges of planetary magnetic index shown with the scintillation boundary determined by Kersley et al.⁶ from 40-MHz transmissions from the BE-B satellite.

regions¹². Which has led to studies of the spectrum of ionospheric irregularities particularly in the F-region from *in-situ*¹³ and ground-based measurements¹⁴. As far as we are aware, the observations reported here represent the first cases of scintillation fading due to F-region irregularities for frequencies in the microwave band at mid-latitudes.

verification is central to our discussion, and we shall henceforth consider equations (1) and (2) as firmly established.

Using a few basic ideas of the theory of general relativity, we can show that a force analogous to a centrifugal force acts on the ions and electrons in a conductor in a gravitational radiation field. Consider a one-dimensional system along the x axis. The metric for plane wave gravitational radiation moving in the z direction^{4,5} is

$$ds^2 = dt^2 - (1 + h(t-z))dx^2 \quad (3)$$

(We use units in which $c = 1$, and assume the radiation has '+' linear polarisation.) The radiation field, h , is dimensionless and constitutes a very small perturbation on the flat-space metric. In this metric field, a freely-falling test particle experiences no coordinate acceleration, and thus two test particles at rest and separated by coordinate distance dx will remain so⁵. On the other hand the measurable physical separation, given by^{5,8}

$$l = (1+h)^{1/2}dx \simeq (1+h/2)dx \quad (4)$$

will change as a function of time, and the two test particles will experience a relative acceleration. On this basis, we may associate a Newtonian tidal force with the radiation field, equal to the mass of a test particle times its acceleration referred to a fiducial test particle. From equation (4) this force is given by

$$F = m\ddot{h}dx/2 \simeq m\ddot{h}l/2; \ddot{h} \equiv d^2h/dt^2 \quad (5)$$

It is clear that this result must be general, and the tidal force will act on all particles, freely falling or not.

To visualise the effect of this gravitational tidal force on the ions and electrons in a conductor, it is convenient to consider a conductor whose centre ($l = 0$) is in free fall. Then a force ($\frac{1}{2}m\ddot{h}l$) will act on an ion at distance l from the centre, and in complete analogy with equation (2), an electric field E and associated voltage V must result

$$E = \Lambda m\ddot{h}l/2e; V = \Lambda m\ddot{h}l^2/4e \quad (6)$$

We must emphasise the theoretical foundation of this result. It rests entirely on equation (2), which we consider to be experimentally established, and on equation (5). If gravitational radiation exists, it is hard on dimensional grounds alone to conceive of a gravitational theory in which an equation analogous to (5) does not hold. Equation (6) would thus seem to rest on equally firm ground.

We can estimate the voltage V in equation (6) for some radiation sources studied by ref. 9. For the Crab pulsar a very rough estimate is $h \sim 10^{-27}$ at a frequency $\omega = 2\pi(60) \text{ Hz} = 377 \text{ Hz}$; then with m taken as the mass of a copper ion and l as 2 km we obtain a very small value of $\sim 10^{-23} \text{ V}$. For a typical supernova in the Virgo cluster of galaxies $h \sim 10^{-21}$ at a characteristic frequency of $\omega \sim 6 \text{ kHz}$; with $\ddot{h} \sim h\omega^2$ we then obtain a more optimistic value of $\sim 10^{-15} \text{ V}$. Such supernova occur about once a month. Supernovae in our Galaxy would give a voltage ~ 3 orders of magnitude greater, but are of less interest since they occur about only once a century.

It is amusing to speculate very briefly on the qualitative design of a practical antenna based on these ideas. To obtain the voltage differences calculated above over a small distance we might consider two conductors of different metals many kilometres in length, the voltage difference at the ends then being proportional to the difference in the values of Λ for the two metals. It would presumably be preferable to use superconductors to minimise thermal and other spurious noise, and a superconducting cover has the added advantage of being a perfect shield against external electromagnetic radiation.

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Palaeozoic and Mesozoic age trends for some ring complexes in Niger and Nigeria

OVER 40 individual granitoid ring complexes exhibiting similar petrological and geochemical variations have been recognised in Nigeria¹. They range in size from 1,500 km² to < 2 km². The ring complexes form part of a more extensive chain along the ninth meridian, extending from the north of the Air Massif through southern Niger and northern Nigeria to the north-western margin of the Benue Trough in Nigeria (Fig. 1). In

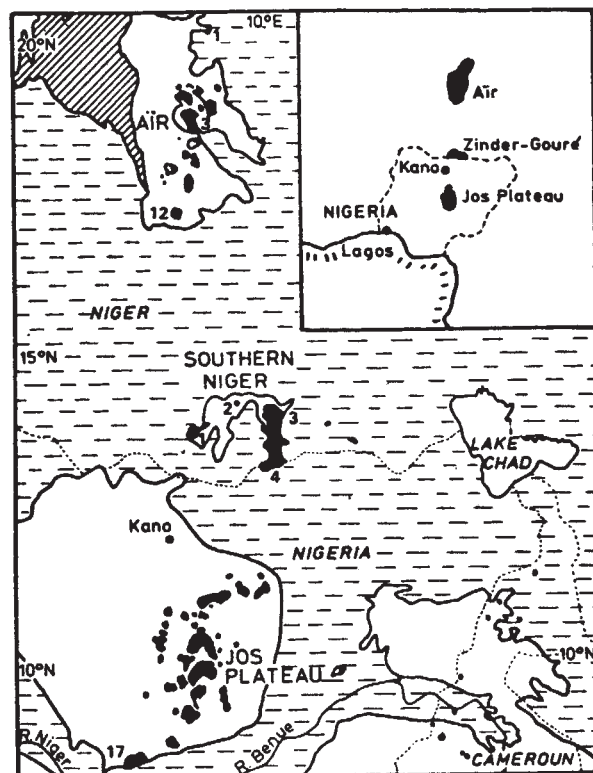


Fig. 1 Distribution of the Palaeozoic and Mesozoic ring complexes in Niger and Nigeria. The identification numbers of individual complexes are taken from ref. 1: 1 Air (mid-Palaeozoic); 1 Adrar Bous, 3 Tamgak-Enfoud group, 12 Tarraouadji; southern Niger (Upper Palaeozoic); 1 Zinder, 2 Tchouni-Zarniski, 3 Gouré, 4 Matsena; Nigeria (Mesozoic); 17 Afu. Shading: clear, Precambrian 'Basement'; diagonal, Palaeozoic; horizontal, Cretaceous-Recent.

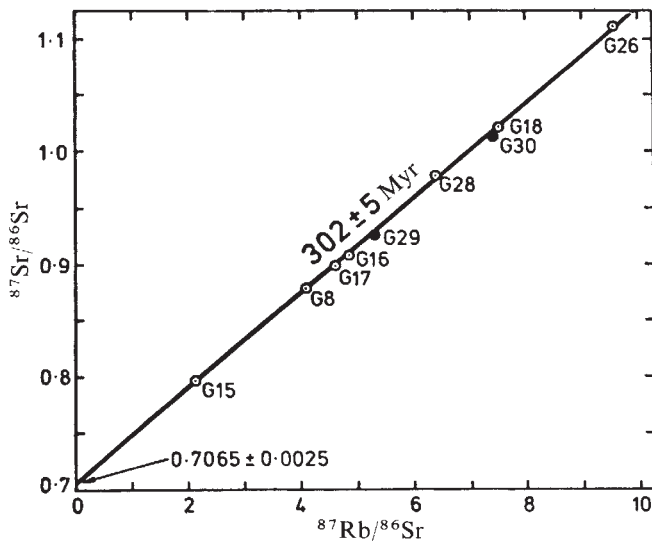


Fig. 2 Rb-Sr whole-rock isochron for the Gouré complex, Mounio Massif, southern Niger. Open circles with G numbers 8-28 represent samples from Gouré. Two additional samples G29 and G30 shown by closed circles, were collected from Adoumchi 20 km south of Gouré airport. $\lambda = 1.39 \times 10^{-11} \text{ yr}^{-1}$.

general the suite of Nigerian complexes are concentrated in a 200-km wide north-south zone and centred around the Jos Plateau. We have previously² investigated the group of ring complexes forming the central zone of anorogenic magmatism in Nigeria, and found a southerly decreasing age trend from early Jurassic to mid-Jurassic times. We give here further evidence for this trend, and point out that its recognition may be useful in ascertaining the drifting motion of the African continent.

The radiometric evidence² we found was consistent with cross-cutting relationships of individual complexes, but the validity of any systematic age sequence in chains of igneous ring complexes has been questioned³⁻⁵. We wish to draw attention to the following observations. New geochronological data for Zaranda, a syenite-related granitoid complex in northern Nigeria⁶, yields an age of 190 ± 10 Myr. In contrast the Afu complex, which represents the southernmost Nigerian ring structure exposed beneath the Cretaceous-Recent sedimentary cover, has yielded a mean age of 144 ± 2 Myr. This range of dates indicates that the individual granitoid ring complexes were not emplaced into the upper crust at the same time (150 Myr

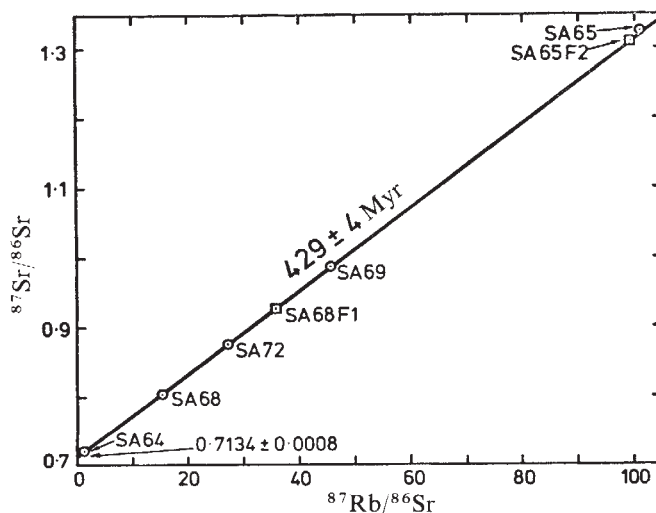
ago) but evolved during the Upper Triassic and Jurassic. Many of the complexes display a complete magmatic cycle, and evidence suggests that the development of an individual centre was established ≤ 5 Myr and then migration continued.

There are two anorogenic ring complex sub-Provinces in Niger represented geographically by southern Niger and by the Air Massif. The southern Niger region¹, consists of the complexes of Zinder, Tchouini-Zarniski and the southerly trending Mounio Massif from Gouré to Matsena (Fig. 1), a series of overlapping centres similar to the Sara-Fier Complex in Nigeria⁷, together with several isolated centres further to the east. Seven subvolcanic granites and porphyries from the Gouré Complex plot on a concordant isochron (Fig. 2) corresponding to an age of 302 ± 5 Myr. Two samples from Adoumchi, the subsequent centre south of Gouré, plot slightly below the isochron suggesting a younger age of ~ 295 Myr. The granite at Zinder yields an age of 330 ± 6 Myr. The age-range for the southern Niger region remains to be confirmed in detail, but it is reasonable to conclude that anorogenic magmatism took place between 340 and 290 Myr ago, and that this period should be regarded as chronologically different from the younger period of Nigerian anorogenic magmatism. On the chronostratigraphic time scale⁸, the ring complexes of southern Niger are Carboniferous in age, and therefore in spite of petrological and geochemical similarities, these structures are considerably older than the equivalent centres in Nigeria.

The ring complexes in the Air Massif¹ are generally more peralkaline than their counterparts in Nigeria, with sub-alkaline biotite granite absent or only a minor component in most of them. A further contrast is provided by the occurrence of massive coarse-grained anorthosite, in several complexes, and olivine gabbro, at Adrar Bous in the extreme north of Niger⁹. Six of the ring complexes lie in a southerly trending zone 250 km long, but there is a pronounced central concentration of overlapping complexes, the Tamgak-Enfoud Group (Fig. 1). Previously, the Air ring structures were believed to have been emplaced during the same interval as the 'Younger Granite' magmatism in Nigeria, although subsequent estimates based on common lead⁹ and K-Ar (ref. 10) methods gave inconsistent dates between 200 and 300 Myr. Preliminary new Rb-Sr whole rock and mineral age determinations for Adrar Bous and Enfoud give dates of 429 ± 4 Myr and 430 ± 15 Myr respectively. The concordant isochron for Adrar Bous is shown in Fig. 3. If this age is accurate for the granites at Adrar Bous, it means that anorogenic magmatism commenced in the Air region during early Silurian times. Stratigraphic evidence at Adrar Bous does not support that, however: the ring complex intrudes and metamorphoses sedimentary rocks which have been assigned a Lower Devonian age⁹. Nonetheless, if our age data are accepted, it means that the anorogenic granites in the north and central areas of the Air Massif are mid-Palaeozoic in age and are chronologically different from their counterparts in southern Niger and Nigeria. An unpublished age (~ 380 Myr) for Tarraouadji, the southernmost complex in the Air Massif (G. Marinelli, R. Black, personal communication) suggests that the Air ring complexes evolved during the Silurian and Devonian periods.

Accepting the concept of sublithospheric heat sources for mid-plate magmatism¹¹, the chains of ring structures in the Niger-Nigerian Province may be useful in charting the continental drift of Gondwanaland before fragmentation. According to Briden¹², there were quasistatic intervals during the Palaeozoic and Mesozoic eras when the Gondwana palaeomagnetic pole positions were stationary for varying lengths of time. Although there is some disagreement regarding the exact chronostratigraphic timing of the palaeomagnetic pole groupings in the Upper Palaeozoic, an earlier Middle-Palaeozoic quasistatic interval is recorded¹³ which coincides with the evolution of the ring complexes in Air. The systematic age range for the Nigerian ring complexes also falls within a later Mesozoic quasistatic interval. Most workers accept that actual polar wandering does not normally occur, but that the continents

Fig. 3 Rb-Sr whole-rock and mineral isochron for the Adrar Bous complex, Air Massif, northern Niger. $\circ$, Whole rocks (SA) $\square$, alkali feldspar separates (F1 and F2). $\lambda = 1.39 \times 10^{-11} \text{ yr}^{-1}$.



themselves have drifted rapidly during palaeomagnetic pole shifts and have remained either stationary or moved slowly during quasistatic intervals. It is perhaps significant that in the Niger–Nigerian Province there exists three groups of granitoid ring complexes which could, with more geochronological evidence and palaeomagnetic measurements, provide a complete record of the movement of the African continent from the beginning of the Silurian to the end of the Jurassic. The age trends of the ring complexes represent the intervals during the Palaeozoic and Mesozoic eras when Africa moved slowly; the absence of intervening ring structures indicating that Africa drifted rapidly.

The long time span from Adrar Bous (429 Myr) to Afu (144 Myr) compared with their small degree of latitude separation is, however, at variance with the palaeomagnetically predicted amount of drift over a single fixed point heat source.

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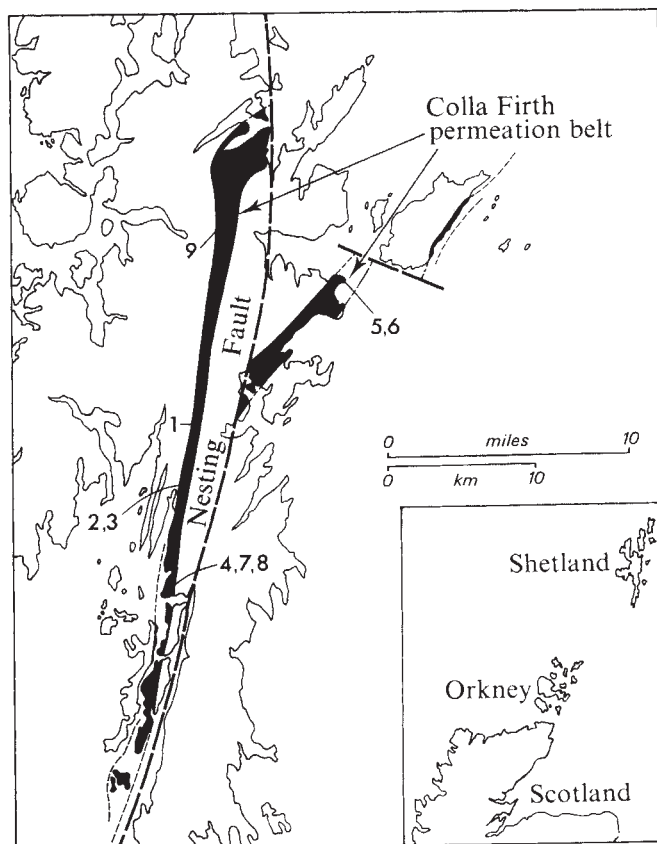


Fig. 1 Location of specimens.

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Age of the migmatisation in the Dalradian of Shetland

WE have dated samples of schistose granite from Shetland using Rb–Sr methods. This provides further evidence for the early phase of Caledonian high grade metamorphism and migmatisation (the Grampian event) which has been detected all the way from western Ireland to northern Norway.

The Colla Firth Group, part of the East Mainland Succession of Shetland, is predominantly composed of steep to vertically-dipping regionally metamorphosed felspathic sandstones with a thickness of up to 4 km (refs 1, 2). These rocks have been correlated with the middle Dalradian of Scotland^{2,3}. They contain a conformable belt of gneiss 1–2 km wide called the Colla Firth permeation belt¹ which can be followed from the southern end of Shetland for ~ 50 km to the north where it is intersected by the Nesting fault and offset 16 km to the south. The offset part of the belt can be followed for a further 20 km to the north-east before it disappears into the sea (Fig. 1). K–Ar ages of ~ 420 Myr have been obtained from nearby rocks⁴.

Along most of the length of the belt the regionally metamorphosed rocks have been changed from semipelites and psammities to gneisses, characterised by streaks of coarse quartz and feldspar, and to rather homogeneous granitoid gneisses. Along its entire exposed length the belt contains frequent intrusive sheets of non-schistose pegmatite and of schistose granite. In places it contains much larger masses of the schistose granite a kilometre or more across. All of these intrude both the gneisses and the adjacent unmigmatized rocks. It has been argued elsewhere that the migmatite belt was formed after the tectonising regional metamorphism of the adjacent rocks, this latter metamorphism being the earliest one discernable in the Colla Firth Group^{2,3}. According to this interpretation, the schistosity in the granite veins and the tectonic fabric of the gneisses are the result of late movements accompanying the migmatization. May⁵ has claimed, however, that the schistosity in the granite veins and the schistosity in the regional metamorphic rocks are the same, so that the migmatization and the regional metamorphism must have occurred at the same time.

To determine the age of migmatization, eight whole rocks samples of schistose granite were analysed for Rb and Sr.

Table 1 Analytical data

Sample number	Rb(p.p.m.)	Sr(p.p.m.)	⁸⁷ Rb/ ⁸⁶ Sr	⁸⁷ Sr/ ⁸⁶ Sr
1	219.3	147.4	4.31	0.7540
2	238.9	135.4	5.22	0.7597
3	187.8	164.0	3.41	0.7460
4	190.0	164.9	3.43	0.7416
5	102.1	243.9	1.21	0.7313
6	162.0	193.1	2.43	0.7402
7	209.9	133.8	4.54	0.7555
8	248.6	54.99	13.24	0.8206
9	171.9	172.1	2.90	0.7376

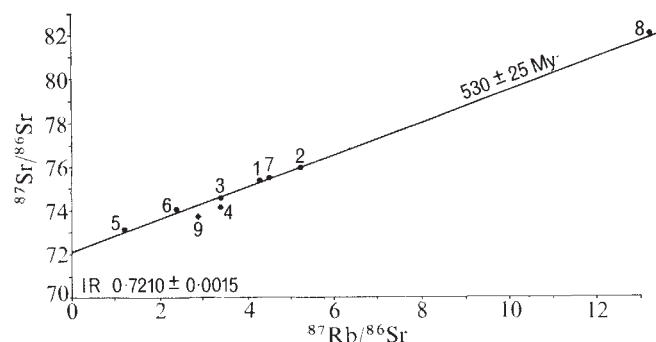


Fig. 2 Rb/Sr whole-rock isochron.

They are all composed of quartz, muscovite, plagioclase, microcline and biotite. Specimen 9 is from a granite gneiss intermediate in nature to the schistose granites and the migmatite gneisses. It was included to determine whether it could be correctly classed as a schistose granite.

Following preliminary analyses by X-ray fluorescence to determine Rb and Sr contents for sample selections and for spiking purposes all samples were analysed by standard isotope dilution techniques and the results of these analyses are given in Table 1 and plotted in Fig. 2. Sample 9 is not included in the isochron calculation which, using York's method II⁶, yields an age of 530 ± 25 Myr with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7210 ± 0.0015 . This age and all other Rb-Sr ages quoted are calculated using $\lambda^{87}\text{Rb} = 1.39 \times 10^{-11} \text{ yr}^{-1}$; the errors given are all 2σ . Considering the distribution of the data points, it is possible that sample 4 is associated with 9, and that 8, because of its relatively high Rb/Sr ratio, has a different source. As no geological or petrographical evidence could be found to support these possibilities they have not, however, been excluded. In fact, the ages obtained by excluding various selections of these data points from the isochron calculation all fall within the lower uncertainty limit of the preferred value given above.

The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7210 is in accordance with a crustal melting source for the schistose granite veins and thus with their derivation from the gneisses of the permeation belt. The age of 530 ± 25 Myr for the schistose granite veins is thus believed to be a good estimate of the age of the permeation belt as a whole. If May⁵ is correct this is also the age of the main phase of the regional metamorphism, but it is more likely that this took place earlier.

This age of 530 ± 25 Myr for migmatization in Shetland is in complete agreement with ages determined for this event in Connemara, 520 ± 30 Myr (refs 7, 8) and North Norway, 530 ± 35 Myr (refs 7, 9). In Scotland, Bell's¹⁰ determination of the age of the Ben Vuirich granite has been recalculated by Dunning⁷ to be 511 Myr. This granite is about the same age as adjacent migmatites in middle Dalradian rocks, the Duchray Hill Gneiss, according to H. J. Bradbury (personal communication). The age of the schistose granite from Shetland agrees with ages for the main phase of high grade metamorphism in the other parts of the Caledonian fold belt.

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Simultaneous measurements of H_2O , NO_2 and HNO_3 in the daytime stratosphere from 15 to 35 km

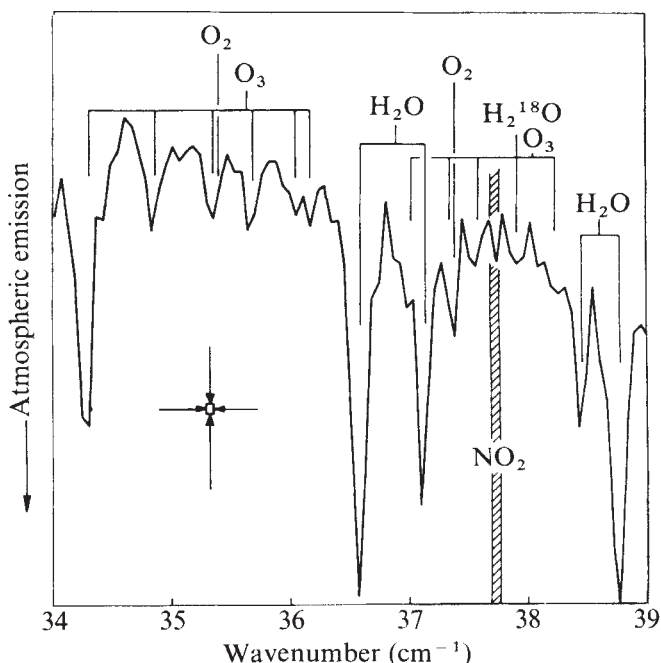
USING Fourier transform spectroscopy in the far infrared with a Michelson interferometer, and helium-cooled bolometric detectors, it is possible to study the emission spectrum of the stratosphere at high resolution (0.05 cm^{-1}) in the spectral range $5-45 \text{ cm}^{-1}$ (see refs 1-3).

We report here initial results from two daytime high altitude balloon flights undertaken during the past year, using far infrared Michelson interferometers, to measure concentrations of several trace gases to altitudes of about 35 km. The aim of the work was to investigate the concentrations of several gases simultaneously, using the wide bandwidth and high resolution capabilities of the Fourier technique. It is possible to obtain information on H_2O , O_3 , HNO_3 , N_2O and NO_2 in one scan of the far infrared interferometer. Results on H_2O , HNO_3 and NO_2 only are presented here, measured at 1200 LT.

The flights were made from the Centre National d'Etudes Spatiales (CNES) balloon launching facility at Aire-sur-l'Adour in south-western France, on September 12 and September 20, 1974. Launches were achieved at about 0900 LT, and observations at altitude (34-36 km) were made during the local noon period.

The experimental system was similar to that used in previous aircraft experiments, though with several modifications and improvements to accommodate the more

Fig. 1 Portion of a far infrared emission spectrum obtained while floating at 35 km, at a zenith angle of 92° . The curve is an average of five runs each taking 8 min to record. The spectral resolution is 0.07 cm^{-1} (unapodised). Shaded line, range of possible centre wavenumbers for the NO_2 Q branch, as judged from theoretical and experimental sources, arrows, resolution width and signal-to-noise ratio (estimated from reproducibility of different spectra).



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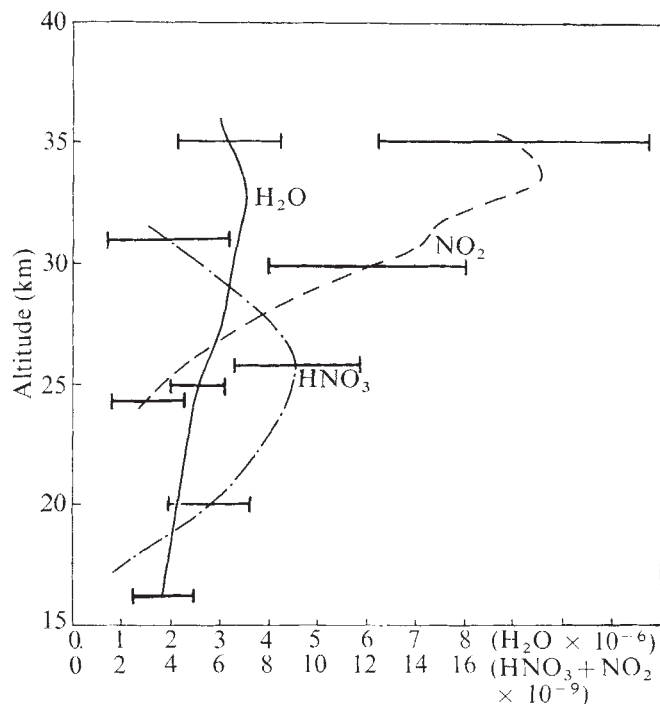


Fig. 2 Vertical profile of mixing ratio for H_2O , HNO_3 and NO_2 , measured during the flight of September 20, 1974 at 44°N 0°W . Uncertainties given in the text. Mass units are given for H_2O and volume units for NO_2 and HNO_3 .

difficult and remote operating conditions encountered on a balloon flight. A full description will be given elsewhere, but it should be noted that limb scanning was used to build up a vertical profile of concentration while the package floated at ceiling. The instrument was stabilised against motions of the gondola by reference to the solar elevation angle which was measured in real time: these motions proved to be very small (amplitude less than 0.1°). A helium-cooled InSb electron bolometer was used.

Figure 1 shows a portion of a typical spectrum, for the range $34\text{--}39\text{ cm}^{-1}$, at a spectral resolution of 0.07 cm^{-1} . Features seen are attributable to H_2O , O_2 , O_3 and NO_2 (refs 2–6); other features attributable to these gases, and to HNO_3 and N_2O , occur at lower wavenumbers.

The results of the September 1974 flights for H_2O , HNO_3 and NO_2 between 15 and 35 km are shown in Fig. 2. It is an important feature of our technique that the profiles were measured simultaneously; the measurements were made during the period $\pm 1\text{ h}$ around noon on September 20, 1974. The uncertainties are typically $\pm 15\%$ for H_2O , $\pm 25\%$ for HNO_3 , and $\pm 35\%$ for NO_2 , and are attributable to uncertainties in radiometric calibration, line intensity measurement, observation angle, and spectroscopic parameters such as integrated line strength.

The results for H_2O show a gradual increase in volume mixing ratio from about 3.0 p.p.m. (by volume) at 15 km to about 5.5 p.p.m. at 33 km. For NO_2 we found about 4 parts per 10^9 at 25 km rising to about 18 parts per 10^9 at 33 km, with slight evidence of a maximum at this level. In the case of HNO_3 a pronounced maximum occurs at about 25 km with mixing ratios of about 9 parts per 10^9 .

The water vapour result agrees well with previous measurements made from aircraft^{2,3} and with data obtained from an earlier single balloon flight over the UK⁷. We have not previously observed such a marked increase of mixing ratio with altitude as is shown in Fig. 2, however, though several measurements support the occurrence of somewhat more humid layers of air above 30 km (refs 8, 9 and 16) in some cases with mixing ratios up to 10–20 p.p.m. There remains a considerable degree of

variability between results above about 30 km, however, and it must be accepted that our understanding of the humidity of the upper stratosphere is far from complete. It should be noted that the apparent increase with height observed in the present results is significant in terms of the relative uncertainty attributable to random errors at different altitudes, since most of the uncertainty indicated in Fig. 2 arises from systematic effects.

In the case of nitric acid, our results agree well with those of Murcay *et al.*¹⁰, who at mid-latitudes have reported volume mixing ratios around 5 parts per 10^9 volume at about 23 km. The extensive data reported by Lazrus *et al.*¹¹ seem to be consistently lower in absolute value than our own results and those of Murcay, however. A nitric acid profile suggested by Fontanella *et al.*¹², for a similar latitude to that at which our measurements were taken shows a lower peak (nearer 20 km) but absolute values of mixing ratio similar to our own.

Turning to nitrogen dioxide, there have been a large number of measurements in the past two years of this species, with some disparity between the different results. Murcay *et al.*¹⁰ report values ranging from 1.5 parts per 10^9 at 20 km to 5 parts per 10^9 at 28 km. Fontanella *et al.*¹² report values of about 1 part per 10^9 at 20 km and 0.3 parts per 10^9 at 15 km. The last two sets of data refer to sunrise or sunset conditions since the solar absorption/limb-sounding method has been used in each case, and the exact timing of the measurements relative to local sunrise/sunset is not available. It is important to identify this relative time very accurately since it is now known that the concentration of NO_2 varies rapidly at dawn and sunset (some recent data have been obtained on the variability of NO_2 at sunrise from our own measurements, and these will be published shortly). Farmer *et al.*¹³ have also reported NO_2 measurements using the solar absorption technique, and obtained about 1.8 parts per 10^9 between 15 and 20 km.

Daytime, as well as early morning and evening measurements of NO_2 have been reported by Brewer *et al.*¹⁴. They report values of around 50 parts per 10^9 at 30 km at mid-day, but Johnson¹⁵ has reinterpreted the data and has obtained values nearer 10 parts per 10^9 , with only slightly lower values at dawn. Results obtained by Chaloner *et al.*¹⁶ show midday profiles ranging from about 6 parts per 10^9 at 20 km to 15 parts per 10^9 at 35 km.

Clearly, therefore, there is a range of results; at 25 km for example, values vary from about 1 to 10 parts per 10^9 . Our own value here is ~ 3.5 parts per 10^9 . In measurements as difficult as this, however, it is in our view reassuring that such close agreement is obtained, and, certainly natural variations must be allowed for in assessing the various results.

Further balloon flights have been made, in April 1975, which spanned sunrise. The results of these flights will be reported shortly, and contain information on the diurnal variability of NO_2 .

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Search for correlation between geomagnetic disturbances and mortality

It is generally accepted that the Sun affects the Earth's magnetosphere and ionosphere by variations in the ultraviolet and X-ray radiation and in the solar wind due both to short lived disturbances such as flares¹ and to long lived effects such as that associated with the 'sector structure' of the interplanetary magnetic field². There have, however, been repeated claims^{3,4} for well over a century that the Sun influences many other processes on Earth, including terrestrial weather and human disorders. Friedman, Becker and Bachman⁵⁻⁷ have presented evidence for an association between geomagnetic storms, cosmic-ray flux variations and psychological behaviour. The term geomagnetic storm is used for worldwide fluctuations in the Earth's field with a scale of about 100γ over a period of several hours, caused by the impact of a solar plasma front on the magnetosphere. A number of Russian scientists⁸⁻¹⁰ have claimed that there is a real association between geomagnetic storms and the incidence of various human diseases. Within this general area, one of the most active areas of current research seems to be the correlation of solar activity and myocardial infarction and stroke. We have searched for a similar correlation in the USA but have failed to find one.

Gnevyshev and Novikova¹⁰ refer to the work of several other groups, published in Russian and unavailable to us. Seventeen papers are referenced, all of which are said to suggest the possibility of direct effects of solar activity on living organisms. They present evidence of two types: first, the results of direct experiments in which biological systems were placed in artificially induced electromagnetic oscillations and second, studies of correlation between geomagnetic indices and mortality due to heart disease and stroke, using the method of superposed epochs and comparison of averaged medical data on days of different geomagnetic activity. The results presented seem to indicate positive correlations, but no estimates of statistical significance are given. We therefore performed similar statistical analyses using US data. Daily numbers of deaths due to specific causes were obtained from the National Center of Health, referring to the actual date of death rather than the registration date.

Geomagnetic activity is measured by magnetic indices. Local *K* indices are based on the measured amplitude of variation over a 3-h interval; corrections for local effects are applied to give world *Ap* indices. Another form of geomagnetic activity is due to hydromagnetic emissions which are enhanced after geomagnetic storms and produce ultra low frequency (~ 1 Hz) fluctuations in the terrestrial magnetic field. This type of activity is measured by a local *W* index which represents the number of 15-min intervals in a 24-h day that contain hydromagnetic emissions.

We compared daily numbers of deaths in the USA due to coronary heart disease and stroke for 1962-66 with the corresponding *Ap* indices using three methods. Mortality data were normalised to remove weekly and seasonal variations and a long term secular trend, since we were looking for short term correlations with natural events. This was done by renormalising with respect to average yearly and weekly curves for the 6-yr period and a linear ramp obtained by least-squares fitting. Mortality and geomagnetic data are shown in Fig. 1.

Our first method was to calculate the correlation coefficient r_k between daily deaths D_i and A_i , the daily value of *Ap*, where $i(=1, \dots, N)$ enumerates days. In the formula

$$r_k = \frac{\sum_{i=1}^{N-k} (A_i - \bar{A})(D_{i+k} - \bar{D})}{(N-k)\sigma_A\sigma_D} \quad (1)$$

$k(>0)$ represents the number of days *D* lags *A*; $k<0$ indicates a lead; $\bar{A}$, $\bar{D}$ are means of the time series; σ_A , σ_D are the standard deviations. The standard error of r_k is given by

$$\sigma_{r,k} = (N - |k| - 1)^{-1/2}(1 - r_k^2) \quad (2)$$

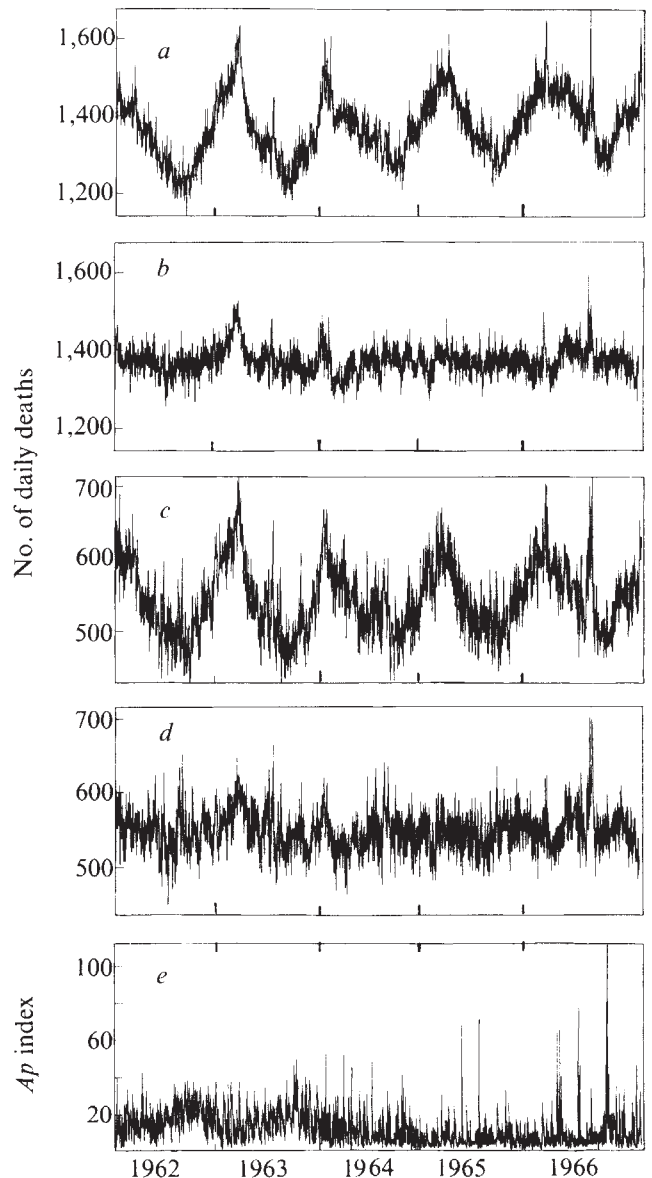
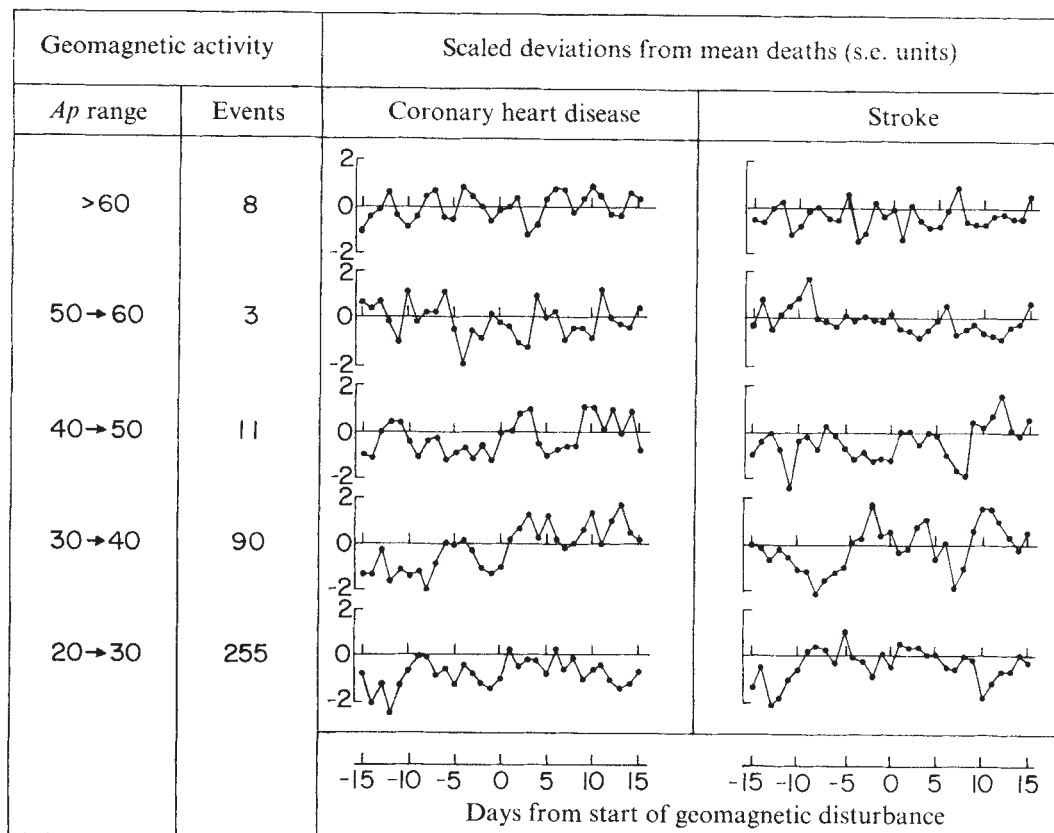


Fig. 1 *a*, Daily US mortality from coronary heart disease, 1962-66. Averaged deaths: Sunday, 1489.1; Monday, 1551.3; Tuesday, 1493.9; Wednesday, 1487.9; Thursday, 1489.6; Friday, 1504.9; Saturday, 1527.3. *b*, Normalised data obtained from *a*. Averaged deaths are the same for each day of the week and equal to 1507.1. *c*, Daily US mortality from stroke, 1962-66. Averaged deaths: Sunday, 549.3; Monday, 555.8; Tuesday, 547.8; Wednesday, 546.4; Thursday, 543.0; Friday, 547.1; Saturday, 552.3. *d*, Normalised data obtained from *c*. Averaged deaths are the same for each day of the week and equal to 549.1. *e*, Geomagnetic *Ap* index, 1962-66.

Fig. 2 Superposed epoch diagrams for mortality due to coronary heart disease and stroke for the period 1962–66. An event occurs when A_p on day zero lies in the ranges given. Mean daily deaths for the total sample and standard deviations (σ) about the mean: coronary heart disease 1507.1, 57.9, stroke 549.1, 27.3. Departures from the mean are measured in units of $\sigma/\sqrt{n}$ where n is the number of events.



Results for $-14 \leq k \leq 14$ are given in Table 1. We found no evidence for correlation at the 3σ significance level, a criterion we used throughout the analysis. For nearly all k , the correlation coefficient is negative, indicating that a small, long term anticorrelation exists. This effect, which is not relevant to our study, arises from low frequency trends in the data.

As an alternative procedure, we constructed superposed epoch diagrams for deaths on days with A_p in selected ranges. Results are shown in Fig. 2, plotted as deviations from the mean daily deaths of the total sample. The deviations were scaled by $\sqrt{n}/\sigma$ where n is the number of superposed events and σ is the standard deviation from the mean of the total sample, so that each plot would have the same width of scatter if the selected subsets were simply a random selection from the total sample. This allows direct comparison of the diagrams and easy detection of statistically significant deviations. In most of the diagrams, the averaged superposed daily deaths throughout the period are less than the total sample mean, another manifestation of the small long term trends already noted. No diagram gives evidence for a statistically significant association between daily deaths and magnetic index.

The third method of presentation was to plot averaged deaths as a function of magnetic index for $-20 \leq k \leq 20$. Again there was no evidence for a significant effect.

Since Gnevyshev and Novikova¹⁰ studied the relationship between mortality levels and geomagnetic indices for individual cities, rather than an entire nation, the above analyses were repeated for 3 metropolitan areas. Daily deaths during 1964 and 1965 due to coronary heart disease in Phoenix, Honolulu and Washington, DC were compared with local K indices. Some results for zero lag are given in Fig. 3, using the third method of presentation. None of the results shows any evidence for correlation.

Finally, we compared daily deaths due to coronary heart disease in San Francisco in 1964 and 1965 with the W index for geomagnetic pulsations. We did this test because Gnevyshev and Novikova attribute their claimed relationship between geomagnetic activity and mortality levels to low frequency fluctuations in the geomagnetic field. Some results for zero lag are given in

Fig. 3. These analyses also showed no evidence for an association.

In summary, our study does not support the findings of Gnevyshev and Novikova, nor their proposal for a new branch of science—heliobiology. It is possible that the correlations they find are either not statistically significant or not due to a causal relationship between geomagnetic disturbances and

Table 1 Correlations between geomagnetic index A_p and daily US mortality from coronary heart disease and stroke, 1962–66

Lag index k	Correlation coefficients, r_k	
	Coronary heart disease	Stroke
-14	-0.061	-0.028
-13	-0.052	-0.037
-12	-0.067	-0.052
-11	-0.063	-0.069
-10	-0.041	-0.040
-9	-0.040	-0.017
-8	-0.030	-0.031
-7	-0.019	-0.039
-6	-0.034	-0.037
-5	-0.040	-0.012
-4	-0.033	-0.039
-3	-0.029	-0.024
-2	-0.052	-0.008
-1	-0.065	-0.021
0	-0.044	-0.018
1	-0.015	-0.037
2	-0.002	0.001
3	-0.010	-0.012
4	-0.025	-0.007
5	-0.023	-0.028
6	-0.014	-0.021
7	-0.028	-0.045
8	-0.019	-0.040
9	-0.009	-0.003
10	0.014	0.003
11	0.001	0.007
12	-0.005	0.004
13	-0.011	-0.007
14	-0.008	-0.012

Deaths lag behind geomagnetic event if $k > 0$, and lead if $k < 0$. Standard error in r_k is constant to 3 decimal places and equal to 0.023.

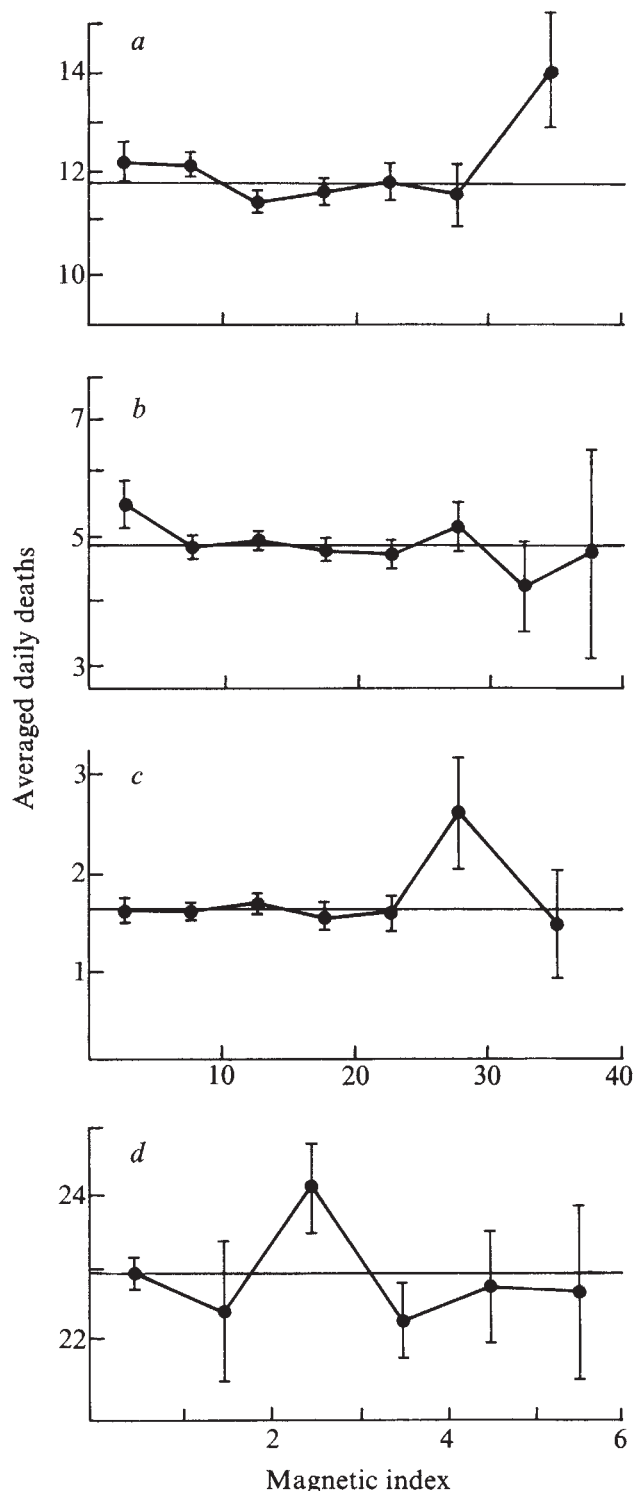


Fig. 3 Averaged mortality due to coronary heart disease as a function of magnetic index for zero lag. *a*, Washington, DC; *b*, Phoenix; *c*, Honolulu; *d*, San Francisco. For *a*, *b* and *c* the magnetic index used was the sum of local *K* indices for the day; *d* the local *W* index was used.

coronary heart disease and stroke. If their correlations are indeed indicative of a causal relationship, it will be necessary to determine whether it is sensitive to geographical location, to phase of the solar cycle or to some other parameter which might distinguish the samples we have analysed from those of the Soviet scientists.

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Hardy-Weinberg ratios and rare male mating advantage

PETIT¹ and others have shown that in a variety of *Drosophila* species the mating success of male genotypes is often dependent on their frequency in the population. This frequency-dependent mating effect seems to be relatively unimportant in females, and by favouring rare male genotypes represents a possible mechanism by which genetic variation can be maintained in a population. Whether or not this form of selection is widespread in natural populations is unknown, although, in the laboratory at least, it has been shown that the phenomenon occurs in insects other than *Drosophila*^{2,3}.

Can this type of selection be detected in the wild without resorting to mating experiments? Unfortunately precise mathematical analysis of the problem, even at the simplest level, is complex and requires the choice of specific functions to describe how male mating success changes with gene or genotype frequency⁴. A more general approach can, however, be adopted, which, while not providing exact answers, highlights two possible problems in detecting frequency-dependent mating effects in natural populations.

Consider a single autosomal locus segregating *n* alleles in a large population of some sexual species. Assume that the generations are separate and that all *n* alleles are maintained in the population by frequency-dependent male mating effects. Other selective forces are assumed to be equal over all alleles. Let the mating success of a male of genotype *ij* be denoted by the function *F*_{*ij*} (which may be dependent on any number of the allele frequencies), the frequency of that genotype be *g*_{*ij*} and the frequency of allele *i* be *p*_{*i*}.

The frequency of each genotype in the next generation can now be calculated by considering the two populations of gamete types. The frequency of each allele in the pool of eggs is unaffected by selection but in the pool of sperm the frequency of allele *i* will be

$$p_i^s = (g_{ii}F_{ii} + \sum_k g_{ik}F_{ik}/2)/\bar{w}$$

where *i* ≠ *k*, and $\bar{w}$ ensures that the sum of all frequencies is one. The genotype frequencies in the next generation are given by

$$g_{ii}' = p_i p_i^s \quad (1a)$$

$$g_{ij}' = p_i p_j^s + p_i^s p_j \quad (i \neq j) \quad (1b)$$

At equilibrium the new genotype frequencies are the same as the old and substituting (1a) and the equivalent relationship

for the homozygote j/j into (1b) yields the set of equilibrium equations

$$\hat{p}_i \hat{p}_j \hat{g}_{ij} = \hat{p}_i^2 \hat{g}_{jj} + \hat{p}_j^2 \hat{g}_{ii} \quad (2)$$

Given the formula relating an allele frequency to its constituent genotype frequencies and that both allele and genotype frequencies sum to one, then the equilibrium form (2) defines the Hardy-Weinberg ratios. Thus, in the conditions of this model, selective forces acting on the sexual success of only one sex cannot be measured, or even suspected, by looking for deviations from the Hardy-Weinberg ratios in a population at, or close to, genetic equilibrium.

A second approach, possible when females are fertilised by only one male per brood, is to assess the frequency of different matings. In particular, consider the mating of the two genotypes i/j and k/l . Provided that not all of i, j, k and l are the same, it is then possible to compare directly the frequency of such crosses when the female is i/j to when the male is i/j . The ratio of these two frequencies gives a direct estimate of the relative fitness of the two males (F_{ki}/F_{ij}).

The direct comparison of mating frequencies can be extended further in populations at genetic equilibrium, since genotype and allele frequencies can then be related by the Hardy-Weinberg result. For example, if the alleles, i, j, k and l are all different then the expected frequency of an i/j female mating with a k/l male is $(2\hat{p}_i \hat{p}_j)(2\hat{p}_k \hat{p}_l)F_{ki}/\bar{w}$ and that for a k/l female mating with an i/l male is $(2\hat{p}_k \hat{p}_l)(2\hat{p}_i \hat{p}_j)F_{il}/\bar{w}$, thus the ratio of these two frequencies again gives a direct estimate of the relative fitness of the two males (F_{ki}/F_{il}). The size and number of groups of matings which can be compared in this way depend on the number of alleles segregating. Table 1 shows examples of the largest groups of matings which can be compared directly for two, three and four or more alleles. Of course, all possible comparisons could be made, but this method has the advantage that expectations are independent of genotype and allele frequencies.

Table 1 Examples of the largest number of direct mating frequency comparisons which can be made in an equilibrium population segregating 2, 3 or 4+ alleles

Number of alleles	Mating ♀ ♂		Expected frequency (with selection)	Expected ratio (no selection)
2	1/2	1/2	$4p_1^2 p_2^2 F_{12}/\bar{w}$	4
	1/1	2/2	$p_1^2 p_2^2 F_{22}/\bar{w}$	1
	2/2	1/1	$p_1^2 p_2^2 F_{11}/\bar{w}$	1
3	1/2	2/3	$4p_1 p_2^2 p_3 F_{23}/\bar{w}$	2
	2/3	1/2	$4p_1 p_2^2 p_3 F_{12}/\bar{w}$	2
	1/3	2/2	$2p_1 p_2^2 p_3 F_{22}/\bar{w}$	1
	2/2	1/3	$2p_1 p_2^2 p_3 F_{13}/\bar{w}$	1
4+	1/2	3/4	$4p_1 p_2 p_3 p_4 F_{34}/\bar{w}$	1
	3/4	1/2	$4p_1 p_2 p_3 p_4 F_{12}/\bar{w}$	1
	1/3	2/4	$4p_1 p_2 p_3 p_4 F_{24}/\bar{w}$	1
	2/4	1/3	$4p_1 p_2 p_3 p_4 F_{13}/\bar{w}$	1
	1/4	2/3	$4p_1 p_2 p_3 p_4 F_{23}/\bar{w}$	1
	2/3	1/4	$4p_1 p_2 p_3 p_4 F_{14}/\bar{w}$	1

In the case of a sex-linked locus, the female population (assumed to be homogametic) is still in Hardy-Weinberg ratios at equilibrium (the males representing the allele frequencies directly). Therefore direct comparisons analogous to those of Table 1 can be made, although the maximum number of matings in each group of comparisons is reduced to two for a pair of alleles or to three otherwise (but note that the number of male genotypes is also reduced).

The use of direct mating comparisons in equilibrium populations can indicate and measure mating selection but the method has the disadvantage of not being powerful enough to dismiss

the possibility of even strong frequency-dependent effects. This is because under such circumstances selective values may be very similar at genetic equilibrium, and thus equilibrium analysis, however detailed, may be fruitless.

The preceding suggests that if frequency-dependent (or indeed constant) sexual selection is acting on one sex to maintain a polymorphism, then, at genetic equilibrium, deviations from Hardy-Weinberg ratios are not expected, but an analysis of the occurrence of specific matings may reveal the selection. Failure to demonstrate selection at equilibrium does not imply that it is not acting or that it is not important. Only the analysis of perturbation experiments can be used to eliminate this form of selection as an important stabilising influence.

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Energetic cost of locomotion in Australian hopping mice

THE mode of locomotion of the hopping mouse can be compared with that of the kangaroo moving bipedally at speed. Because the latter cannot be monitored due to technical difficulties, I have used the hopping mouse in a study of the energetics of this type of locomotion at speeds of 0.5-7 km h⁻¹. I found that at 2.5-3 km h⁻¹, the stride frequency stabilised, as did steady-state oxygen consumption. Above 5 km h⁻¹ oxygen consumption again increased although stride frequency did not. The result of this pattern was that the costs of hopping in *Notomys cervinus* were markedly below those predicted for a quadruped. The overall pattern had similarities with those displayed by kangaroos¹.

I used three specimens (34-38 g) of *N. cervinus*, the fawn hopping mouse, native to Australia, collected in stony desert in western Queensland. This species can be trained on a treadmill and hops readily. Energy cost was estimated from steady-state oxygen consumption measured for 15-30 min while the animals travelled at speeds of 0.5-7.0 km h⁻¹ inside a Perspex chamber (40×10×15 cm) with a flexible seal at its base. Air was drawn through the chamber at approximately 4 l min⁻¹, and nitrogen, bled into the chamber at a constant rate, indicated that, at all speeds, there was no leakage of air out of the chamber. Oxygen consumption was measured with a Beckman F-3 paramagnetic analyser with a full scale of 20.5-21% oxygen. Air temperature was maintained at 26-28 °C so that shivering did not affect the oxygen consumption at lower speeds—such an effect was apparent in initial experiments at 17-19 °C. Strides were monitored using a video camera with a slow motion playback capability. Twenty sequences of twenty strides were counted for each animal at each speed, and stride length was calculated from stride frequency and treadmill speed.

Oxygen consumption was similar for all three animals, although one had a consistently higher consumption (Fig. 1). Between 0.5 and 2.5 km h⁻¹ oxygen consumption increased linearly with increasing speed. At these speeds the mice either 'trotted' with the hind legs moving independently or 'galloped' quadrupedally with the hind legs

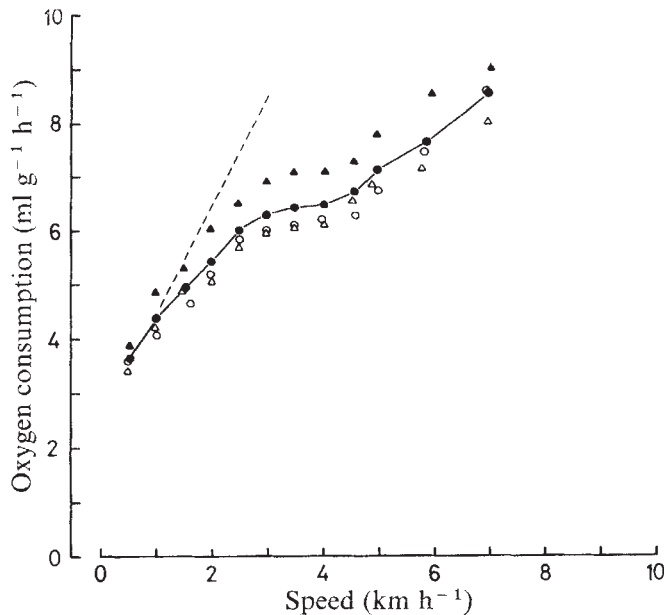


Fig. 1 Steady-state oxygen consumption of the hopping mouse *N. cervinus* as a function of speed. The mean values at each speed (●) are joined by the solid line. Values for individual animals are indicated by different symbols. The dashed line represents the oxygen consumption predicted for a mammal the same weight as the mean weight of the hopping mice, 36.6 g.

moving together. During this phase the energy cost did not diverge markedly from that predicted for a quadruped². An increase in speed in this range was accomplished by increases in stride frequency and length (Fig. 2).

As in the case of kangaroos¹, oxygen consumption reached a plateau at the speeds at which stride frequency became nearly constant, 3 km h⁻¹. This stability in energy cost did not persist, however, although stride frequency remained constant at speeds > 7 km h⁻¹. At > 4.5 km h⁻¹, oxygen consumption again increased with an increase in

speed, in an apparently linear manner, but at a rate well below that predicted for quadrupeds (which is a behaviour we assume kangaroos would also show at their highest speeds).

One difference between the pattern of locomotion of hopping mice and of kangaroos was that there was no immediate shift to complete hopping at the speeds at which stride frequency and oxygen consumption tended to stabilise initially (3 km h⁻¹). More or less continual bipedal hopping was not achieved by the mice until beyond 4.5 km h⁻¹, and the animal with consistently high oxygen consumption did not achieve complete hopping, even at 7 km h⁻¹. The overall picture for the relationship between type of gait and speed was similar for the two animals with the lower oxygen consumption: between 0.5 and 1.5 km h⁻¹ the mice mostly trotted, between 1.5 and 2.5 km h⁻¹ they mostly galloped, and above 2.5 km h⁻¹ some hopping was apparent, initially in the form of a sequence of four to six hops interspaced with galloping. At 3.0 km h⁻¹ galloping comprised 65% of the strides and hopping 35%. The amount of hopping increased gradually until it was continuous at 6 km h⁻¹. (Preliminary tests with other *N. cervinus* suggest that this is the common pattern.) For the third animal trotting did not change to galloping until ~ 2.5 km h⁻¹, and significant hopping was not evident until 6 km h⁻¹. Even at 7 km h⁻¹ hops only comprised 45% of strides. Why this animal should be different from the others was not obvious; initially it was rather recalcitrant, but with training it performed well. Hopping (or saltatorial bipedal locomotion) seems to be associated with a marked shift in the energy costs of locomotion from the patterns established for running quadrupeds² and bipeds³. This results in a much lower overall cost of locomotion in the small hopping mice and lower cost at moderate speeds in the large kangaroos.

Our data demonstrate some of the advantages of hopping, particularly for a small rodent living in a desert where food is sparse and cover for protection from predators is limited. Although reasons may also be suggested for the selective advantage of hopping for kangaroos¹, there is still the question why kangaroos are the only large animals with saltatory locomotion and our experiments give some insight here also. At low speeds the *N. cervinus* largely follow the energy predictions of ref. 2 and when they diverge from this pattern they immediately achieve lower locomotory costs. Kangaroos, however, have much higher costs than predicted for quadrupeds at low speeds, and it is not until moderately high speeds (17–18 km h⁻¹ for an 18-kg animal) that the costs of hopping become less than those predicted.

In an analysis of the mechanical characteristics of very long leaps of kangaroos, Badoux⁴ observed that, to develop the necessary acceleration, the feet must exert a force directly proportional to the animal's mass and inversely proportional to the length of leg. Taylor *et al.*⁵ reiterated that the work involved in lifting 1 g of tissue a given vertical height is the same for small animals as for large, if the mechanical efficiency of the muscle of both animals is the same. Because both the resting metabolic rate and the relative cost of locomotion decrease with increasing weight, larger animals have to increase their metabolism by a proportionately greater amount than do small animals to lift a given weight of tissue a given height. The energetic implications of this for hopping bipeds are obvious, particularly at low speeds when elastic storage of energy would be low. The pentapedal gait of large kangaroos at slow speeds may reflect a partial answer to this problem, since anatomical specialisations limit the use of quadrupedal locomotion.

Howell⁶ has suggested that biped hopping is an extreme form of the gallop. This is possibly illustrated by the data on the mouse with the higher oxygen consumption. While this animal did not commence significant hopping until ~ 6 km h⁻¹, stride frequency and oxygen consumption reached

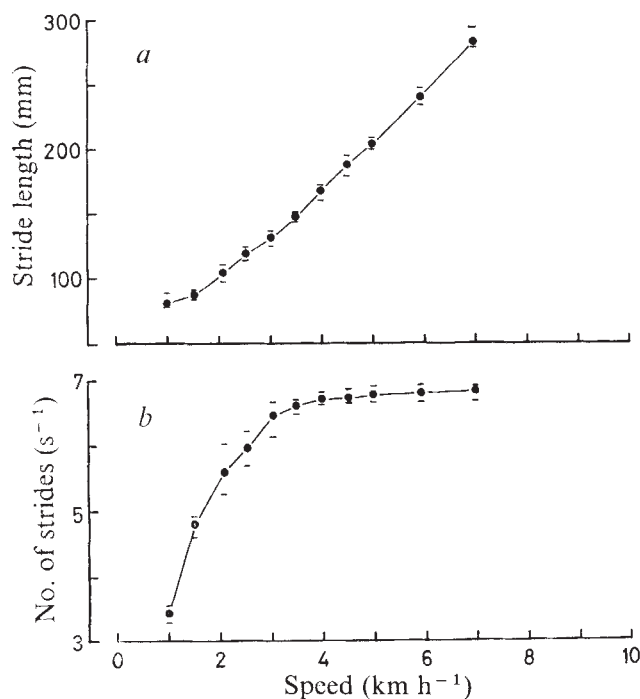


Fig. 2 a, Stride length; b, stride frequency as a function of speed for *N. cervinus* during steady-state locomotion. The values shown in both graphs are the mean values for the three individuals and the horizontal bars indicate the range of values.

their plateaux at the same speed as for the other hopping mice, 2.5–3.0 km h⁻¹. Stride frequency tends to stabilise in most animals once they commence galloping⁷. Consequently it may be that a similar but smaller change in oxygen consumption occurs in other species at speeds above the transition from trotting to galloping, but that this is emphasised in species with anatomical specifications such as those for hopping. This needs further investigation since the data with which Taylor *et al.*² established a relationship between the energetic costs of locomotion and body weight were mostly collected at speeds below the trot–gallop transition. This was particularly the case for their kangaroo rats (*Dipodomys merriami* and *D. spectabilis*).

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Mutants of *Klebsiella pneumoniae* resistant to several antibiotics

SPONTANEOUS antibiotic-resistant mutants that arise in bacterial populations are usually only resistant to the particular antibiotic used in the culture medium used for their isolation. In the experiments described here, however, mutants of *Klebsiella pneumoniae* resistant to chloramphenicol, sodium nalidixate and trimethoprim, three unrelated antibiotics, were obtained by including any one of the antibiotics in the culture medium. Because these multiply resistant mutants were commonly found in the alimentary tract of animals experimentally infected with antibiotic-sensitive *K. pneumoniae* and then fed on antibiotic-containing diets they may be clinically important.

Mutants resistant to antibiotics other than trimethoprim were obtained by spreading 0.75 ml of a 24-h broth culture containing $\sim 5 \times 10^8$ viable organisms per ml over the surface of a well-dried plate of MacConkey's agar containing antibiotic (20 $\mu\text{g ml}^{-1}$). The plates were incubated at 37 °C for 24 h and colonies that grew on them purified by plating on ordinary MacConkey's agar. Washed broth cultures and Sensitest agar (Oxoid, CM409) containing trimethoprim (3 $\mu\text{g ml}^{-1}$) were used for obtaining mutants resistant to this antibiotic. Antibiotic sensitivity tests were performed by placing disks containing streptomycin (25 μg), tetracycline (30 μg), chloramphenicol (30 μg), ampicillin (25 μg), neomycin (30 μg), spectinomycin (30 μg), rifampicin (50 μg), sodium nalidixate (30 μg), sulphafurazole (50 μg), trimethoprim (1.25 μg) and furazolidone (15 μg) on the surface of plates of Sensitest agar spread with well diluted broth cultures; the plates were incubated at 37 °C for 24 h and read.

When mutants resistant to sodium nalidixate (*nal*^r), chloramphenicol (*cmp*^r) and trimethoprim (*tmp*^r) were isolated from 27 of 32 epidemiologically unrelated strains of *K. pneumoniae* belonging to 20 different capsular types, they were found to be resistant not only to whichever of these three antibiotics were incorporated in the selection

medium but also to the other two; 50–200 colonies grew on each of the plates used. All the colonies on the chloramphenicol and trimethoprim plates that had been inoculated with five of the strains were found by replica plating to be *cmp*^r *nal*^r and *tmp*^r. So were all those on the sodium nalidixate plates inoculated with three of these strains; a few of the 50 or so colonies that grew on each of the sodium nalidixate plates inoculated with the other two strains were *nal*^r only, the remainder being resistant to the three antibiotics. Disk tests indicated that the sensitivity of these *cmp*^r *nal*^r *tmp*^r mutants to tetracycline, streptomycin, neomycin, spectinomycin, rifampicin, polymixin, sulphafurazole and furazolidone had not altered appreciably; the strains from which they were derived were resistant to ampicillin. Using very light inocula, six of the multiply resistant mutants were passaged ten times in 10-ml amounts of broth at 37 °C, each passage occupying 24 h; their antibiotic-resistance pattern did not alter as a result. Attempts to transfer the resistance of four of them to *Escherichia coli*, K12 were unsuccessful. The colonial morphology of the mutants was the same as that of their parent strains, and so was the capsular type of all five examined by Dr Ida Ørskov and the virulence for mice by intraperitoneal injection of the only one tested. Like their parent strains, the mutants were prototrophic.

Table 1 Minimum inhibitory concentration of antibiotics for *K. pneumoniae* mutants and their parent strains

Antibiotic	Median MIC ($\mu\text{g ml}^{-1}$) for	
	parent strains	mutants
Sodium nalidixate	3	100
Chloramphenicol	3	100
Trimethoprim	0.4	25
Tetracycline	3	12
Sulphafurazole	50	100
Furazolidone	0.8	1.5
Streptomycin	1.5	1.5
Neomycin	1.5	1.5
Spectinomycin	12	12
Rifampicin	25	25
Polymixin	0.8	0.8

Ten mutant and ten parent strains were examined. The minimum inhibitory concentration (MIC) was determined by inoculating small areas of plates containing twofold falling concentrations of antibiotic in Sensitest agar with approximately 200 viable organisms and then incubating the plates at 37 °C for 24 h. The lowest concentration of antibiotic that prevented growth was recorded as the MIC.

The minimum inhibitory concentrations of sodium nalidixate, chloramphenicol and trimethoprim for the mutants were 25–50 higher than those for their parent strains (Table 1). Those of tetracycline, sulphafurazole and furazolidone, but not of streptomycin, neomycin, spectinomycin, rifampicin and polymixin, were slightly higher for the mutants than those for their parent strains.

Mutants resistant to streptomycin, spectinomycin and rifampicin were isolated from three *K. pneumoniae* strains; they were resistant only to the antibiotic used in their isolation.

To determine whether a multiply resistant *K. pneumoniae* flora could emerge *in vivo* during the administration of only one antibiotic, groups of 15 1-d-old chicks were given orally 10^8 viable organisms of a spectinomycin-resistant mutant of a *K. pneumoniae* strain and then fed freely on diets containing sodium nalidixate, chloramphenicol or trimethoprim (100 or 500 mg kg⁻¹) or no antibiotics for 10 d. Cloacal swabs taken at intervals from the chicks were each smeared over the surface of a MacConkey plate and a Sensitest plate, both containing spectinomycin (20 $\mu\text{g ml}^{-1}$), and a sodium nalidixate disk and a chloramphenicol disk were placed on the MacConkey plate and a trimethoprim disk was placed on the Sensitest plate. The plates

Table 2 Faecal excretion of antibiotic-resistant organisms by chicks fed diets containing chloramphenicol, trimethoprim or sodium nalidixate after receiving orally a culture of *K. pneumoniae* sensitive to these antibiotics

Antibiotic administered	Time after commencement of administration	No. out of 15 chicks excreting <i>K. pneumoniae</i> organisms that were		
		only the antibiotic administered	resistant to chloramphenicol trimethoprim and sodium nalidixate	all sensitive
Chloramphenicol	4	0(0)	4(13)	11(2)
	8	0(0)	9(10)	4(1)
	17*	0(0)	1(5)	7(3)
Trimethoprim	4	3(8)	7(4)	6(5)
	8	12(11)	7(6)	1(0)
	17*	14(9)	1(1)	1(0)
Sodium nalidixate	4	0(0)	0(0)	15(15)
	8	0(0)	0(0)	13(8)
	17*	0(0)	0(0)	15(15)
None	4	0(0)	0(0)	15(15)
	8	0(0)	0(0)	15(15)

Chicks received antibiotic in a concentration of 100 mg kg⁻¹ and (figures in parentheses) 500 mg kg⁻¹.

*Seven days after the feeding of antibiotic-containing diets ceased.

were incubated at 37 °C for 24 h and those with colonies of *K. pneumoniae* organisms on them that were resistant to sodium nalidixate, chloramphenicol and trimethoprim were recorded. These organisms were found in the faeces of many of the chicks fed diets containing chloramphenicol or trimethoprim (Table 2); from some of them they were the only kind isolated. Organisms of the infecting strain resistant to trimethoprim only were also common in the faeces of most of the chicks in the trimethoprim group. The multiply resistant organisms were also isolated from a few of the chicks in the chloramphenicol and trimethoprim groups 7 d after withdrawing the antibiotic-containing diets; at this time most of the chicks in all the groups had no or very few organisms of the infecting strain in their faeces. No resistant organisms of this strain were found in the faeces of any of the chicks fed diets containing sodium nalidixate or no antibiotics. Surprisingly, on account of the ease with which they can be isolated *in vitro*, no *nal^r E. coli* were found in the faeces of chicks in the sodium nalidixate group.

Antibiotic-resistant mutants of enterobacteria other than *K. pneumoniae* that were available in this laboratory were examined for resistance to antibiotics different from the ones used in their isolation. In addition, those whose parent strains were prototrophic were cultured on a medium consisting of (g l⁻¹) K₂HPO₄, 7; KH₂PO₄, 3; (NH₄)₂SO₄, 1; MgSO₄·7H₂O, 0.1; NaCl, 5; glucose, 5; agar, 15 to determine whether they too were prototrophic. The antibiotic that had been used to isolate the mutants from different pathogenic and non-pathogenic strains of *E. coli* of human, bovine, porcine and fowl origin was sodium nalidixate (106), streptomycin (40), ampicillin (14), spectinomycin (10) and rifampicin (1), from different *Salmonella* strains was sodium nalidixate (24), streptomycin (18), ampicillin (1), spectinomycin (3) and rifampicin (5) and from different *Shigella flexneri* and *S. sonnei* strains was sodium nalidixate (8), streptomycin (5), spectinomycin (1) and rifampicin (2). All these 238 mutants were only resistant to the antibiotic used in their isolation and all the 214 with prototrophic parents were also prototrophic.

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Selective entry of immunoblasts into gut from intestinal lymph

THE observation¹ that the lymphoid blast cells in the thoracic duct lymph of rats homed preferentially to the lamina propria of the small gut after being injected intravenously has received ample confirmation²⁻⁴. But although the thoracic duct lymph of rodents comes principally from the intestine there must also be a significant contribution from other viscera as well as from the caudal somatic lymph nodes. It follows that the large lymphoid immunoblasts present in thoracic duct lymph may have come from several sources, and it has been shown that their numbers increase after antigens have been injected subcutaneously into the hindquarters⁵. Are the immunoblasts that home to the small gut selected randomly, or do they have some special feature which mediates their extravasation in the gut? Straightforward immunological factors such as the specificity of the immunoblasts or the presence of antigens in the gut do not seem to be of primary importance^{6,7}.

Immunoblasts teased from somatic lymph nodes cannot home to the gut nearly as well as those from mesenteric nodes or thoracic duct lymph^{2,8} but the performance of immunoblasts which normally enter the lymph from somatic nodes has not been measured. By cannulating appropriate lymph ducts in experimental sheep we have studied the problem directly and our results are reported here.

Yearling sheep were anaesthetised and polyvinyl cannulae inserted into the intestinal lymph duct and an efferent duct of one of the peripheral, somatic lymph nodes (popliteal, prefemoral or prescapular). After the operation the conscious sheep were placed in metabolism cages and the lymph was collected quantitatively into sterile, heparinised, plastic bottles⁹⁻¹¹. In these relatively young sheep up to 15% of the white cells in the intestinal lymph were immunoblasts, but in the efferent lymph from somatic nodes, immunoblasts accounted for 2% or less of the white cells. For this reason 2 ml of a mixture of suspensions of killed bacteria (*Corynebacterium parvum*, *Brucella abortus* and *Salmonella typhi* O and H, Wellcome Research Laboratories) was injected subcutaneously into the drainage area of the cannulated node so that 4 d later, when the immune response was greatest, 20-40% of the cells in the efferent lymph consisted of immunoblasts^{12,13}. An 8-h collection of either intestinal or efferent lymph was then incubated for 1 h at 38 °C with ¹²⁵I-iododeoxyuridine (IUDR, Radiochemical Centre, Amersham) at a final concentration

Table 1 Percentages of injected radioactivity present in tissues of sheep 20 h after injection of autochthonous immunoblasts collected from intestinal lymph or efferent lymph of somatic lymph nodes and labelled *in vitro* with ^{125}I UDR

Type of labelled cells injected	Immunoblasts from efferent lymph of somatic nodes				Immunoblasts from intestinal lymph				
	1	3	5	7	2	4	6	8	10
Experiment no.									
Small intestine	0.9	0.4	0.8	0.6	35.3	30.5	27.8	27.0	28.4
Spleen	16.0	16.8	21.0	19.0	0.2	1.4	0.3	1.4	0.4
Mesenteric lymph nodes	0.1	0.1	0.2	0.1	1.8	3.4	2.7	2.9	3.4
Somatic lymph nodes	1.3	2.6	0.9	0.8	0.2	1.1	0.3	0.7	0.6

of $0.1 \mu\text{Ci ml}^{-1}$. The total number of white cells in the collections varied between 10^9 and 10^{10} and included 10–40% immunoblasts; autoradiographs showed later that 70–80% of the latter had labelled nuclei. The labelled cells were then washed once and returned to the sheep by intravenous injection. The sheep was killed 20 h later and selected organs were removed, cut into small pieces and loaded into counting vials so that the gamma emission of each organ could be measured in a scintillation spectrometer. The relevant results from nine such experiments are shown in Table 1.

Clearly immunoblasts generated in somatic nodes did not enter the small gut but tended to localise in the spleen and, incidentally, did not reappear in significant numbers in samples of either lymph or blood. Conversely, immunoblasts generated in the lymphoid tissue associated with the gut avoided the spleen and localised mainly in the small gut; up to a further 10% of the injected cells were recovered in the intestinal lymph during the course of the experiments. The latter observation shows that, as in studies on rats^{3,4,6} most radioactivity remained associated with intact immunoblasts throughout the experiment. Unfortunately, it is not practical to prepare representative autoradiographs from the major organs of an adult sheep, and so little can be said about the microanatomical localisation of the labelled cells—we assume that it is generally similar to that found in the rat^{3,4}.

It was suggested, on *a priori* grounds, that immunoblasts which enter the gut must be IgA secretors⁴ and there is evidence for this view^{8,14}. In the experiments reported here we found, by immunodiffusion tests, that detergent extracts of washed cells from intestinal lymph always contained IgA as the major immunoglobulin. Extracts of lymph cells from stimulated somatic nodes contained mainly IgG, with some IgM, but we found no IgA. Furthermore, the immunoblasts from somatic nodes did not acquire the ability to home to gut even when they had been collected and incubated in isologous intestinal lymph plasma, which is rich in IgA¹⁵.

These findings support the view that one of the intrinsic cellular factors which mediate the extravasation of immunoblasts in the small gut is the production of IgA or a molecule associated closely with it. Certainly the view³ that lymph-borne immunoblasts from any source may extravasate into the gut is wrong in any general sense.

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Protection of mice against *Babesia* and *Plasmodium* with BCG

Babesia spp. are haemoprotozoans, and some cause diseases of considerable economic importance in cattle. Complete protection against challenge has been recorded only in animals recovered from artificial¹ or natural infections with a virulent strain of the organism. We have achieved the same result with *B. microti* and *B. rodhaini*, two of the species which parasitise mice, by previous infection with *Mycobacterium bovis* strain BCG (Bacillus Calmette-Guerin). Evidence is provided that neither antibody specific for surface antigens of the parasites nor increased phagocytic capacity is responsible for this protection. We suggest that BCG protects by increasing the release of non-antibody soluble mediators of immunity postulated to cause the death of *B. microti* inside red cells². Preliminary observations on protection against *Plasmodium berghei yoelii* and *P. vinckei* are also presented.

Eight female CBA mice 6 weeks old were given 2×10^7 live BCG (Glaxo strain) intravenously. Two weeks later 10^6 *B. microti* were injected intraperitoneally into these mice and into an equal number of controls. In the mice that received BCG a few parasites were seen in tail blood smears after about a week, but none thereafter during an observation period of more than 100 d. The control group showed the usual primary infection curve for this parasite (Fig. 1a).

Subsequently a group of nine 6-week-old female CBA mice were given 10^6 *B. microti* intraperitoneally 28 d after they had received 2×10^7 live BCG intravenously. For 155 d no parasites were observed in smears from these mice, even though further challenges of 2×10^6 , 3×10^6 , 2.5×10^7 and 10^6 organisms were given 14, 25, 50 and 100 d, respectively, after the initial infection. Indeed, absence of recrudescence after splenectomy of three mice on day 40, and failure to transfer infection with the spleens from these animals, suggest that a sterile immunity was achieved.

All of 57 mice, including males, have now been completely protected in eight experiments. Eight of these mice withstood 7.5×10^8 organisms. This is a massive dose compared with challenges usually given in protection experiments with blood parasites^{3–6} and indicates the strength of

the protection conferred by BCG. The dose used produced a 2% parasitaemia within hours of being given intra-peritoneally, but the count did not rise further. As healthy parasites became fewer over the next 2 d the inclusion bodies shown by electron microscopy to be degenerate intraerythrocytic parasites² appeared. These were in sufficient numbers to account for at least most of the

original healthy parasites. Thus *B. microti* die inside the red cells of mice protected with BCG.

Indirect fluorescent antibody titres to *B. microti* antigen were negligible in sera from mice given BCG 26 d previously and not challenged with *B. microti*, and also in serum from mice protected with BCG (Table 1). This test detects all antibody specific for surface antigens of the parasite. Furthermore, giving 3 mg silica intravenously (Doröntrop Quartz No. 12, $<5 \mu\text{m}$) either before or after challenging two groups of six BCG-immunised mice with *B. microti*, which would be expected to decrease antibody production and phagocytic activity, respectively⁷, did not decrease the protection that BCG gave. Thus this protection is unlikely to be due to cross-reacting antibodies, or enhancement of either antibody production or phagocytic activity. In any event the intraerythrocytic degeneration of the parasites precludes their death inside macrophages.

Ten female CBA mice in two experiments were completely protected against *B. rodhaini* (Antwerp Strain), which is invariably fatal in unprotected mice, by giving them live BCG intravenously 3 weeks before challenge (Fig. 1c). When protection of one of these mice lapsed temporarily from day 20 we obtained light microscopical evidence of intraerythrocytic death of *B. rodhaini* also. Again we used 2×10^7 BCG and 10^6 parasites. This number of organisms killed the four control mice on day 9.

Similarly, in one preliminary experiment we were able to protect four out of four female CBA mice against 10^6 *P. berghei yoelii* (Strain 17X) by intravenous injection of 2×10^7 live BCG 1 month beforehand (Fig. 1d). Some male CBA mice (two out of four) were less well protected. An appreciable degree of protection, again with light microscopical evidence of intraerythrocytic death of parasites, has also been achieved in eight mice with *P. vinckei* in the same conditions (Fig. 1e). This parasite is normally lethal in mice, and killed the four controls on day 8, but the protected group survived.

Vaccines incorporating killed *Mycobacterium* sp. and parasites, given intradermally, subcutaneously or intramuscularly, have been used against *B. argentina*³ and *P. knowlesi*^{6,8,9}. All except the one containing merozoites⁶ were only moderately successful. Killed *Mycobacterium* sp. alone has not been protective, perhaps because it was never given intravenously. Live BCG, on the other hand, has been reported to protect against some protozoa, the success varying with the route of administration. Thus intraperitoneal live BCG has been unable to protect mice against *Trypanosoma cruzi*¹⁰ or *Toxoplasma gondii*¹¹. Likewise, live BCG injected into the retrobulbar space of rabbits did not protect the eye from *Toxoplasma gondii*¹². In contrast, pre-treatment with intravenous live BCG reduced the number of circulating *Trypanosoma cruzi* in mice¹³ and protected rabbits' eyes from *Toxoplasma gondii*¹². In neither case, however, was the protection as strong as that which we have observed against *B. microti*, *B. rodhaini*, *P. berghei yoelii* or *P. vinckei*. This may be simply a question of timing and dose of BCG rather than any fundamental difference between the protection afforded against the different protozoa.

BCG provides nonspecific resistance in a wide variety of diseases, both neoplastic^{14,15} and infectious^{16,17}. In certain

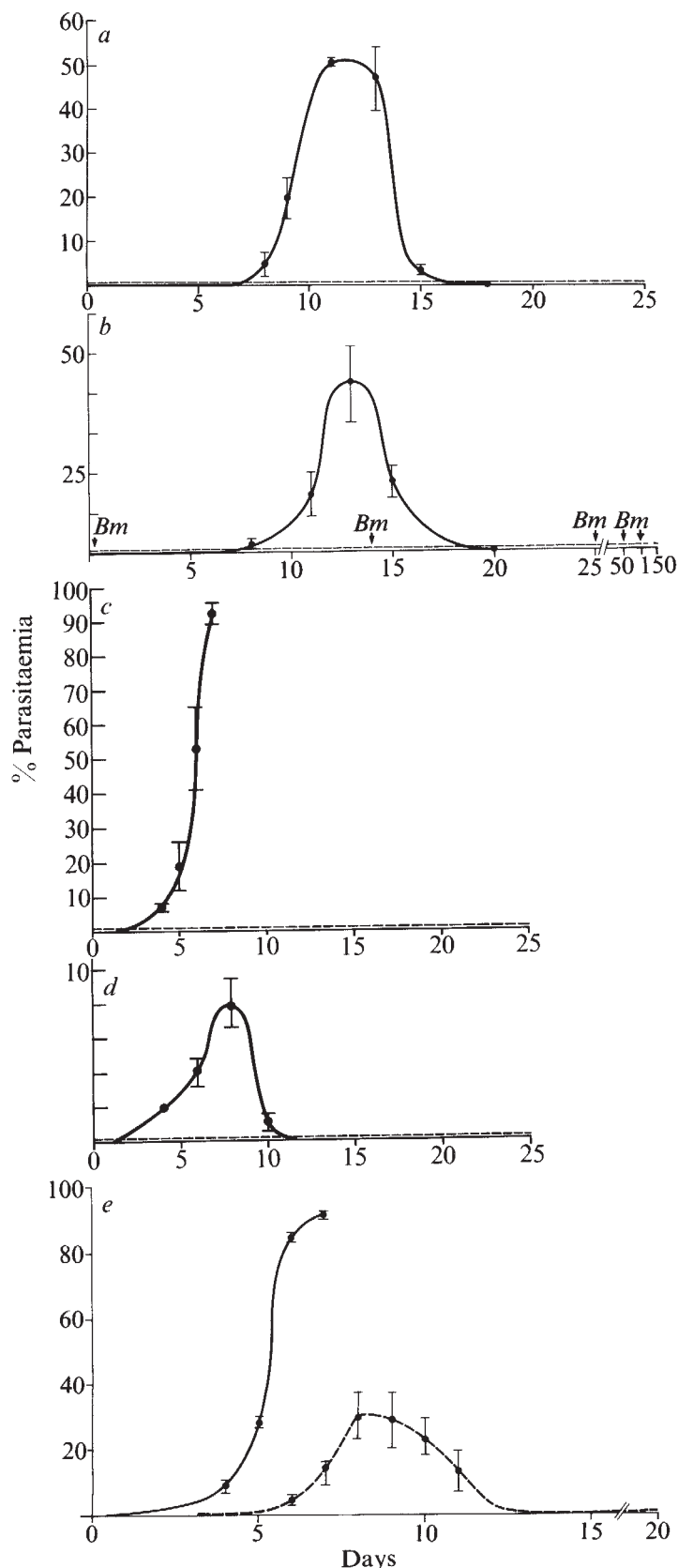


Fig. 1 a, Course of infection of mice given 2×10^7 live BCG intravenously, and 10^6 *B. microti* 14 d later. — — —, BCG-vaccinated; —, non-vaccinated. b, 10^6 *B. microti* given 28 d after 2×10^7 live BCG intravenously. Bm, unsuccessful rechallenges; — — —, BCG-vaccinated; —, non-vaccinated. c, 10^6 *B. rodhaini* given 21 d after 2×10^7 live BCG intravenously. — — —, BCG-vaccinated; —, non-vaccinated. d, 10^6 *P. berghei yoelii* given 28 d after 2×10^7 live BCG intravenously. — — —, BCG-vaccinated; —, non-vaccinated. e, 10^6 *P. vinckei* given 28 d after 2×10^7 live BCG intravenously. — — —, BCG-vaccinated; —, non-vaccinated. s.d. as shown.

conditions BCG can increase antibody production¹⁸ and make macrophages hyperactive¹⁹. From our evidence it seems unlikely that either of these two mechanisms, or cross-reacting antibodies, can explain the protection observed. The occasional appearance of parasites in smears from all the mice infected intraperitoneally with *B. microti* 14 d after BCG inoculation and in some of those protected against *B. rodhaini* eliminates the possibility that activated peritoneal macrophages²⁰ prevented the parasites reaching the circulation.

Immunity to *Babesia* is not yet understood. Thymus-derived lymphocytes are necessary for recovery from infection^{21,22}, but their role is uncertain. Transfer of large volumes of recovery serum have yielded inconsistent results^{4,23-25}; thus it seems unlikely that T lymphocytes merely help in the production of antibody that is free in the serum. Furthermore there is no evidence that T lymphocytes can kill *Babesia* parasites or infected erythrocytes by contact lysis.

Table 1 Anti-*Babesia* antibody levels

Time serum taken	Titre
26 d after BCG alone	0
12 and 20 d after <i>B. microti</i> (BCG 14 d before <i>B. microti</i>)	1:20
14 d after <i>B. microti</i> (BCG 28 d before <i>B. microti</i>)	1:20
Recovered controls	1:2,560

Mice given BCG, BCG and *B. microti*, and *B. microti* alone. Antibody levels detected by an indirect fluorescent antibody test. Serum from at least six mice pooled for each reading.

Another hypothesis is that T lymphocytes are necessary for the release of a non-antibody mediator of immunity. Indeed, the massive T-cell activation in spleens of mice infected with *P. berghei* yoelii²⁶ could be explained on this basis, as the authors remarked. There are many such products of activated lymphoid cells which exhibit various activities *in vitro*; some, for instance, cause intracellular death of *Toxoplasma gondii*²⁷, and others of *M. tuberculosis*²⁸.

Among these mediators are the substances with interferon and migration-inhibition activities present in the serum of BCG-infected mice after stimulation either specifically or with certain unrelated antigens²⁹. As well as protecting mice from *M. tuberculosis*³⁰, these substances can inhibit various unrelated organisms *in vitro*³¹. The finding that BCG must be given intravenously for appreciable release of the mediators of this inhibition³⁰ is consistent with the requirement for this route of administration to protect against certain protozoa¹⁰⁻¹³, and our failure to protect with subcutaneous BCG. Our results are also in keeping with the original proposal by Mackaness³² that cellular immunity, although specifically induced, may be non-specifically expressed; he also suggested that this could be accomplished by migration inhibition factor³³. Furthermore, interferon activity has been reported in the serum of mice infected with *P. berghei*³⁴ and interferon inducers protect mice against this organism^{35,36}.

For these reasons, and in view of the intraerythrocytic death of parasites both in normally recovering² and BCG-protected mice, we consider that the most likely mechanism of immunity to these two genera of parasites in mice is the release of such a mediator, the output of mediator(s) being increased, and thus protection augmented, by previous infection with BCG.

The duration and details of the nature of this protection remain to be established. Nevertheless, in addition to giving a greater understanding of the basic mechanism of immunity involved, this approach may lead to a practical method of stimulating immunity against these two genera

of blood parasites. Further, its nonspecificity promises activity against a range of species and strains.

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Contact-induced spreading is a new phenomenon depending on cell-cell contact

THERE is firm evidence that the characteristic changes of cell behaviour *in vitro* described as contact inhibition of locomotion¹, contact inhibition of extension² and contact inhibition of phagocytosis³ occur as a direct result of cell-cell contact. In addition it has been suggested⁴ that the inhibition of both pinocytosis⁵ and blebbing⁶, in those regions of the margins of cells in contact with other cells, may also be a direct result of cell-cell contact. These examples may well be part of a general class of cell-contact-dependent phenomena which could play an important role in cellular interactions. I report here another contact-dependent reaction which takes place between chick embryo pigmented retina epithelial (PRE) cells in culture. In brief, it can be shown that the degree of polarisation exhibited by these cells and the degree to which they are spread on their substrate is influenced profoundly by whether or not they are in contact with other cells.

Observations⁷ of cultured PRE cells moving on a plane substrate have shown that collisions between the cells usually result in the development of stable contacts between the cells, so that islands and sheets of cells are eventually formed from a culture which consisted initially of isolated

single cells. In addition it has been found that, whereas isolated PRE cells lacking contacts with other cells are usually only poorly spread on the substrate and only infrequently display fibroblast-like leading lamellae, those PRE cells incorporated into islands of cells are much more extensively spread and display typical leading lamellae if marginally positioned in an island⁷. My preliminary observations suggested that as a previously isolated and poorly spread cell made contact with another cell or island of cells, it underwent a striking change in morphology and behaviour to resemble the cells of the island it had joined⁷. The investigation reported here confirms this observation and demonstrates that this change in morphology is dependent on cell-cell contact.

A single-cell suspension of 10-d chick embryo PRE cells was prepared and plated out on to collagen-coated coverslips as previously described⁸. The cultures were incubated at 37 °C for 5 h to allow the cells to attach to the substrate before being prepared for photography. A field of cells was selected and photographed at 30-min intervals for periods of up to 6 h. A selection from a representative series of such photographs is shown in Fig. 1.

The characteristic difference in morphology between isolated cells and cells in contact with other cells is clearly seen in Fig. 1a. The isolated cell illustrated is clearly only poorly spread on the substrate and as a result its nucleus is obscured by pigment granules. The general appearance of such isolated cells suggests that they are 'unpolarised' in that they usually lack a fibroblast-like leading lamella and they display vigorous and apparently uncoordinated blebbing activity at their margins. Although the cells are poorly spread they clearly adhere relatively well to the substrate since cell detachment was never observed although all observations were made on inverted cultures. In addition I have found that such cells can withstand vigorous washing

and perfusion without detaching from the substrate (unpublished observations). By contrast to the isolated cells, those incorporated into islands are much more extensively spread on the substrate (Fig. 1a). Cells that are marginally positioned in an island exhibit clear 'polarisation' in that they display typical fibroblast-like leading lamellae from those parts of their margin not in contact with other cells. Blebbing by these well spread cells is only very rarely observed.

The sequence of photographs shown in Fig. 1 demonstrates the striking change in morphology shown by an isolated cell after colliding with an island of cells. The first signs of this change are evident within 30 min of the collision (Fig. 1b). By this time the cell has begun to spread more extensively on the substrate and as a result its nucleus has become less obscured by pigment granules, blebbing has decreased and the first signs of polarisation are evident. Within 90 min of the collision the cell closely resembles the other cells making up the island, blebbing has ceased, the cell is well spread on the substrate and a leading lamella is beginning to develop from the area of its margin not in contact with other cells (Fig. 1c). With further time the cell becomes indistinguishable from its neighbours in the island (Fig. 1d).

Table 1 Comparison of the area of substrate occupied by single cells and cells in two-cell islands

	Mean cell spread area $\pm$ s.e. (μm^2)	<i>n</i>	
Single cells	315.4 $\pm$ 27.5	25	
*Two-cell islands	496.2 $\pm$ 38.8	25	<i>P</i> < 0.001

*The area of complete two-cell islands was measured and the mean area occupied by the individual cells was obtained by division.

Five cultures of PRE cells were prepared essentially as described in the legend to Fig. 1, but the original cell suspension was adjusted to $3.2 \times 10^5 \text{ ml}^{-1}$ before being plated out. Medium, substrate, culture vessels, sample size and incubating conditions were as described in the legend to Fig. 1. The cultures were incubated for 24 h and then the coverslips with attached cells were removed, fixed with formol saline and stained with Harris' haematoxylin. The areas of five single cells and five two-cell islands in each of the fixed cultures was measured using the Curtis¹⁰ modification of the Chalkley¹¹ random array method. Sufficient estimates of each measured area were obtained to ensure that the final estimate had a standard error of 5% or less.

There seems little reason to doubt that this change in the behaviour and morphology of a previously isolated cell is a direct response to contact with other cells. Alternative explanations in terms of adaptation to culture conditions or recovery from cell dissociation procedures are unlikely since the characteristic differences between isolated cells and those in contact with other cells are maintained for several days in culture. Equally it seems unlikely that the observations can be explained in terms of local diffusion gradients surrounding the islands of cells. Isolated PRE cells move only rather slowly and inefficiently⁷; thus an isolated poorly spread cell may lie within 5–10 μm of the edge of an island of well spread cells yet it will display no signs of spreading or polarisation unless it establishes visible (to the light microscope) contact with the island. This observation could be reconciled with an explanation in terms of diffusion gradients only if such gradients were extremely local in effect. Very short range gradients caused by diffusion boundary layer effects have been proposed to influence the division of cells in culture⁹. Such a system seems unlikely to be operating here, however, since it can be shown that the individual cells comprising a two cell island occupy a significantly greater area of the substrate than do single isolated cells (Table 1). In fact the data in Table 1 indicate that a collision between two previously isolated cells results in both cells increasing by approximately 50% the area of the substrate that they occupy individually. It is difficult to

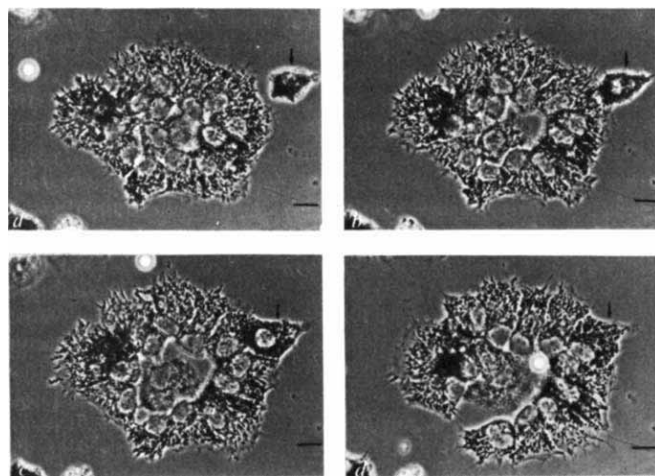


Fig. 1 Contact-induced spreading of a PRE cell. A single cell suspension was prepared from the pigmented retina of 10-d chick embryos as previously described⁸. The cells were suspended in Ham's F10 supplemented with 10% chick serum at pH 7.6 and the concentration was adjusted to $4.8 \times 10^5 \text{ ml}^{-1}$. Samples of 0.2 ml of the suspension were plated out into 9-mm diameter glass rings 1 cm high, attached with sterile silicone grease to collagen-coated coverslips⁸. Cultures were incubated at 37 °C under a gas phase of 5% CO_2 , 20% O_2 and 75% N_2 . After 5 h the glass rings were removed and the coverslips with attached cells were converted to inverted filming slides for photography. Photographs were obtained using a Zeiss photomicroscope fitted with phase contrast optics; the cultures being maintained at 37 °C by an air curtain incubator. a–d, Series of photographs of the same microscope field to illustrate the spreading of a single PRE cell (arrow) after contact with an island of cells. a, After 5 h in culture, arbitrarily defined as 0 time; b, 0 + 30 min; c, 0 + 90 min; d, 0 + 240 min. See text for details. The scale bar represents 10 μm .

see how this response at the single cell contact level could be explained in terms of any diffusion gradient theory.

It seems clear that the marked change in morphology and behaviour shown by isolated PRE cells in response to a collision with an island of cells is dependent on cell contact. Since the most striking aspect of the response is the extensive spreading of the cell I propose that the phenomenon be called contact-induced spreading.

There have been no reports of a reaction similar to contact-induced spreading occurring in fibroblastic cell types but there is at least preliminary evidence to suggest that the phenomenon may not be restricted to PRE cells. Trinkaus¹² noted that while isolated *Fundulus* gastrula cells in culture were normally only poorly spread on the substrate, those incorporated into islands of cells were spread extensively. Additionally, a reaction similar to contact-induced spreading has been observed in cultured chick liver parenchyma cells¹³ and cultured limpet haematocytes (T. A. Partridge, personal communication). It is interesting that all these examples are of cells that assume an epithelioid morphology in tissue culture and it seems possible therefore that contact-induced spreading may be a characteristic of cultured epithelial cells.

The mechanism of contact-induced spreading is obscure but it seems clear that it must involve changes in the adhesion of the cell to the substrate since the area of cell-substrate apposition is considerably increased as a result of the reaction. It seems therefore that the development of a cell-cell contact can affect cell-substrate adhesion. A similar conclusion was reached by Trinkaus from his observations of cultured *Fundulus* gastrula cells¹².

It is likely that contact-induced spreading also involves a change in the locomotory behaviour of the cell since as a result of the reaction a previously unpolarised blebbing cell develops a fibroblast-like leading lamella and, from time-lapse film observations⁷, appears to be attempting to move away from the islands it has joined. It is apparent from Fig. 1 that the phenomenon also involves the development of extensive regions of contact, and presumably therefore adhesion, between the previously isolated cell and its neighbours in the island. Although it has been shown that ultra-structural contact specialisations develop between PRE cells within 24 h in culture⁷, it is not known whether or not these develop rapidly enough to play any role in contact-induced spreading.

Contact-induced spreading seems to be another example of the group of cellular interactions dependent on cell-cell contact. Whether or not the various members of this group share common mechanisms remains to be established. The findings reported here, however, again emphasise the importance of cell-cell contacts in regulating the behaviour of cells, at least *in vitro*.

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Chronic suppression of immunoglobulin allotype production in adult congenic mice

MOUSE allotypes refer to the inheritance of antigenic differences on the constant region of immunoglobulin (Ig) heavy chains (C_H). As such, allotypes are used as genetic markers to distinguish allelic C_H genes, the expression of which is codominant in heterozygous mice^{1,2}. The expression of a paternal C_H gene, however, can be suppressed in heterozygote offspring if the mother has previously been made immune to the allotype encoded by the paternal gene³. Neonatally induced suppression of this sort is known to lead to (or result in) the generation of allotype-specific suppressor T lymphocytes which directly or indirectly prevent allotype production by B lymphocytes⁴⁻⁶. In contrast to this we have shown that allotype suppression can also be initiated in adult mice, that is in BALB/c mice that are congenic for the C57BL allotype (C.B-17 mice)¹. When X-irradiated C.B-17 mice (550 r.) are injected with thymocytes from BALB/c donors immune to the C57BL allotype, IgG_{2a} allotype production in the C.B-17 recipients becomes chronically suppressed⁷. We now report that allotype suppression in C.B-17 mice is mediated by T cells; that production of only the IgG_{2a} class allotype (G^2) is suppressed, presumably as a result of direct interaction between specific T and B cells, and that the capacity of spleen cells to suppress host allotype production can be transferred serially from one C.B-17 mouse to another and as yet without obvious limit.

Mouse 7S Ig allotype determinants of BALB/c ($F^{10}G^{1,6,7,8}H^{9,11}$), of C.AL-20 a BALB/c congenic strain with the allotype of the AL/N inbred strain ($F^{10}G^{4,6,7,8}H^1$) and of C.B-17 ($F^5G^2H^{9,16}$) are denoted here according to the Potter-Lieberman nomenclature where F, G and H refer to the IgG₁, IgG_{2a} and IgG_{2b} subclasses, respectively¹. Anti-allotype immunity was induced in BALB/c, C.AL-20 and C.B-17 donor mice by reciprocal immunisation with each other's Ig in the manner described previously⁸. The recipients in each cell transfer experiment were tested for the presence or absence of different allotypes by means of gel precipitation in micro-Ouchterlony plates.

The kinetics of G^2 suppression in C.B-17 mice that received thymocytes, bone marrow cells or spleen cells from BALB/c mice immune to G^2 are shown in Table 1. After the intravenous injection of 3×10^6 – 5×10^6 thymocytes into X-irradiated C.B-17 recipients (550 r.), G^2 began to disappear as early as 2 weeks later and after 5 weeks was undetectable (groups A and B). Host allotype suppression was chronic and although not shown here, it still persists in C.B-17 recipients 1 yr after cell transfer. The injection of comparable numbers of bone marrow cells (mostly B cells) did not, however, suppress G^2 (groups C and D). Moreover, the exposure of suppressor spleen cells to AKR mouse antiserum specific for T cells (anti- θ serum) followed by the application of complement (C') abrogated the ability of these cells to suppress G^2 production (groups E and F). Spleen cells treated with anti- θ serum gave a good proliferative response (^{125}I -5'-iododeoxyuridine incorporation) to *E. coli* lipopolysaccharide (Bacto 055-B5), a B-cell mitogen⁹, but unlike the control spleen cells, showed only a marginal proliferative response to phytohaemagglutinin (Gibco), a T-cell mitogen⁹ (not shown). These latter results with mitogens were taken as independent evidence for the efficacy and specificity of anti- θ treatment. We conclude that G^2 suppression is dependent on T-cell immunity.

As Table 2 shows, the antigenic basis for host allotype suppression relates solely to the G^2 globulin. First, the involvement of a non-Ig serum antigen is precluded since spleen cells from BALB/c mice that were made immune to chromatographically pure Fc fragments of C57BL/6 7S

Table 1 T-cell-dependent suppression of the IgG_{2a} allotype (G²) in X-irradiated C.B-17 mice after their intravenous injection with BALB/c lymphoid cells immune to G²

Group	No. of immune cells injected*			No. of recipients showing G ² /No. injected† (weeks after cell transfer)					
	Thymus	Bone marrow	Spleen	1	2	3	4	7	20
A	3 × 10 ⁶			5/5		5/5	2/5	0/5	0/4
B	5 × 10 ⁶			18/18	9/18	3/18	0/18		0/16
C		3 × 10 ⁶		6/6		6/6		6/6	6/6
D		5 × 10 ⁶		6/6		6/6		6/6	6/6
E			10 × 10 ⁶	14/14	2/14	0/14		0/14	
F			10 × 10 ⁶ (treated with anti-θ serum)	13/13	13/13	13/13		13/13	

*BALB/c mice were made immune to the IgG_{2a} allotype of C57BL mice (G²) after five or more intraperitoneal injections of C57BL/6 serum hyperimmune to rabbit red cell ghosts; all BALB/c donors were producing precipitating antibody against G² at the time of cell transfer. The AKR mouse anti-θ serum came from Dr J. B. Smith of this institute; exposure of spleen cells to this antiserum was done as follows (group F): 10⁷ cells per ml were incubated for 45 min with a 1/16 dilution of antiserum (in phosphate-buffered saline containing 0.1% bovine serum albumin) at 37 °C in a 5% CO₂ atmosphere. After one wash, the cells were resuspended in a 1/10 dilution of guinea pig complement (1 ml of complement per 10⁷ cells) and incubated for 30 min more at 37 °C. The cells were then washed three times before being injected into C.B-17 mice. Control spleen suspensions were treated as above except AKR normal mouse serum was substituted for AKR anti-θ serum (group E). The results of groups B, E and F represent two or more separate experiments.

†C.B-17 recipients were X irradiated with 550 r. 1 d before cell transfer in the following conditions: 190 kV; 15 mA; filtration, 1 mm Al and 0.5 mm Cu; dose rate 40–45 r. min⁻¹. Recipient sera were reacted with BALB/c anti-G² in micro-Ouchterlony plates to test for the presence of G² allotype.

Ig were able to suppress G² production in C.B-17 mice (group C). Moreover, C.B-17 serum (group B) could substitute for C57BL/6 serum (group A) as an immunogen. C.B-17 serum is not known to be different from that of BALB/c except to contain Ig allotypes of C57BL mice. Second, allotype determinants that characterise the IgG₁ (F^s) and IgG_{2b} (H^s) subclasses continue to be produced in G²-suppressed mice. Evidence of this is deduced from the fact that both anti-F^sG² (groups A–C) and anti-H^s (group D) gave precipitin bands with serum from G²-suppressed C.B-17 mice. It is important to note that the use of C.AL-20 donor mice immune to G² was necessary in group D because the IgG_{2b} molecules of both BALB/c and C.B-17 mice share the H^s determinant. With BALB/c mice as donors, it would be impossible to distinguish H^s production of host cells from that of donor cells. Earlier observations by Jacobson *et al.*⁴ are consistent with the above results as Ig-4b (F^s) was also not suppressed in mice that were suppressed for Ig-1b (G²) production by means of neonatal exposure to anti-allotype serum.

From the above, it seems clear that the IgG₁, IgG_{2a} and IgG_{2b} subclasses can be produced independently of one another. This could mean that B cells independently differ-

entiate into producers of IgG₁, IgG_{2a} or IgG_{2b}, or that C_H genes for the IgG subclasses are expressed sequentially in differentiating immune cells, in which case the expression of IgG₁ and IgG_{2b} must precede that of IgG_{2a}. It also seems clear that BALB/c suppressor T cells must have allotype receptors for G² (G²-suppressor T cells). With G² recognition acting as the stimulus, such T cells might proceed to kill G²-bearing B cells directly, or alternatively, they might elaborate a suppressive factor which specifically prevents B cells from becoming G² producers. A mechanism similar to the latter is perhaps to be favoured in view of recent evidence for T-cell factors that have cooperative^{12,13} or suppressive effects^{6,14} on specific Ig production.

A more indirect mechanism can be postulated: in this the target of G²-suppressor T cells is another T cell that specifically helps B cells produce G² (G²-helper T cell). This would imply that G²-helper T cells carry G² determinants on their surface. To suggest any other basis for discrimination seems unwarranted since the specificity of G² suppression requires immunity to only the allotype determinants located on the Fc fragment of IgG_{2a} molecules (Table 2). We have yet to obtain any evidence for G²-helper T cells.

The long term persistence of allotype suppression

Table 2 Restriction of allotype suppression in X-irradiated C.B-17 mice to the IgG_{2a} allotype (G²)

Group	Donor mice* (7S Ig allotypes)	Donor mice immunised with†	C.B-17 host allotypes (F ^s G ² H ^s , ¹⁶) present 3–5 weeks after intravenous injection of donor spleen cells§ (No. of recipients positive/No. injected)		
			F ^s	G ²	H ^s
A	BALB/c (F ¹⁹ G ^{1,6,7,8} H ^{9,11})	C57BL/6 serum	7/7	0/7	
B	(F ¹⁹ G ^{1,6,7,8} H ^{9,11})	C.B-17 serum	8/8	0/8	
C	(F ¹⁹ G ^{1,6,7,8} H ^{9,11})	Fc fragment of C57BL/6 7S Ig	9/9	0/9	
D	C.AL-20 (F ¹⁹ G ^{4,6,7,8} H ⁴)	C57BL/6 serum		0/8	8/8

*C.AL-20 donor mice are BALB/c congenic mice that carry the Ig allotype of the AL/N mouse strain. These mice were given by Dr Michael Potter of the National Cancer Institute. F, G and H denote the IgG₁, IgG_{2a} and IgG_{2b} subclasses, respectively; the numbers correspond to individual allotypic determinants (nomenclature of Potter and Lieberman¹). Mouse IgG₁, IgG_{2a} and IgG_{2b} have been alternately denoted by Herzenberg *et al.*² as Ig-4, Ig-1 and Ig-3, respectively. Here a lower case letter is used to distinguish allotypes (for example, Ig-1a and Ig-1b) and a second number is used to designate individual allotype determinants (for example, Ig-1.1).

†Donor mice were immunised as described in Table 1 except that the first antigen injection was given in Freund's complete adjuvant. Papain digestion¹⁰ of C57BL/6 7S Ig gave Fc fragments that were purified over DEAE-cellulose according to the method of Fahey and Askonas¹¹.

§C.B-17 recipients were X irradiated with 550 r. 1 d before cell transfer in the conditions described in Table 1. Each recipient was injected intravenously with 20 × 10⁶ spleen cells from the respective donor mice. The antisera used to test for the presence of F^s, G² and H^s in C.B-17 recipients were C.B-17 anti-F^sG², BALB/c anti-G² and C.AL-20 anti-H^s. The indicated specificity of these antisera was assured after testing them against the Igs of various plasmacytomas which were grown in BALB/c, C.B-17 or C.AL-20 mice.

prompted us to transfer serially the spleens of G²-suppressed recipients into other C.B-17 mice. In this way, we intended to evaluate the potential for expansion of T cell-mediated immunity. Of three such experiments attempted, Table 3 shows the results of the one that has been under way for the longest time. Starting with the entire spleen of one BALB/c mouse immune to G², we have suppressed G² production in 78 C.B-17 recipients. This corresponds to 91% of all injected recipients and covers four cell transfer generations and a cumulative time span of 46 weeks.

general context, it is interesting to speculate that allotype-specific suppressor T cells may constitute part of a normal regulatory system which controls the production of different Ig allotypes by B cells.

As far as we know this is the first time that chronic allotype suppression has been initiated in adult mice and in mice with a genetic background different from SJL. The abnormalities of Ig regulation in SJL mice¹⁸ have been considered as a possible explanation for the susceptibility of (SJL×BALB/c) F₁ mice to chronic allotype suppres-

Table 3 Propagation of IgG_{2a} allotype (G²) suppression in X-irradiated C.B-17 mice by serial transfer of spleen cells

Group	Cell transfer generation	Cumulative time (weeks)	No. of C.B-17 mice donors*	used as recipients†	No. of recipients showing G ² /No. injected (weeks after cell transfer)				
					2	3	4	5	6
A	1			6	0/6	0/6			0/6
B	2	9	3	18	18/18	10/18	1/18	0/17§	0/16
C	3	28	2	6	6/6	6/6	6/6	0/5§	0/5
				6TX	6/6	5/6	5/6	5/6	1/6
D	3	35	3	10	10/10	2/10	1/10		0/9§
				10TX	10/10	7/10	6/10		1/10
E	3	36	4†	24	17/24	6/24	1/23§		0/22§
F	4	40	1	6		6/6	2/6		0/6

TX, thymectomised.

*The suppression of G² in C.B-17 mice was initiated with the spleen of one BALB/c mouse immune to G²; the spleen was divided equally as a cell suspension among six C.B-17 recipients (group A). When G² suppression occurred, three of these C.B-17 recipients were used as spleen cell donors for serial transfer into other C.B-17 mice. This kind of procedure was repeated over four cell transfer generations, as indicated. All cell transfers were done intravenously.

†Four of the G²-suppressed mice in group D (non-TX recipients) were immunised exogenously with six injections of C57BL/6 serum before being used as donors. Normal, unirradiated C.B-17 mice served as controls for the hyperimmunisation. These did not produce precipitating antibody to G², neither did the transfer of their spleens into other C.B-17 mice cause G² suppression.

‡All C.B-17 recipients received 550 r. of X irradiation 1 d before cell transfer. Half of the C.B-17 mice in groups C and D were thymectomised 4 weeks before serving as recipients for cell transfer. Recipient sera were reacted with BALB/c anti-G² sera in micro-Ouchterlony plates to test for the presence or absence of G².

§Times at which one or more of the injected recipients were found dead.

Two further points should be made: (1) Exogenous immunisation of G²-suppressed recipients with hyper-immune serum of C57BL/6 mice boosted the potential of these recipients to suppress G² production in other C.B-17 mice (group E); this potential was not yet exhausted at the fourth transfer generation (group F). (2) Thymectomy of prospective C.B-17 recipients delayed the onset of G² suppression (groups C and D). The reason for this is unclear.

Two explanations for the extended serial transfer of G² suppression in C.B-17 recipients seem reasonable. The first is that G²-suppressor T cells of the original donor can be clonally propagated—although with this as the sole explanation, it is difficult to understand why B-cell immunity (antibody responses) cannot be expanded as easily as the present example of T-cell immunity¹⁵. Therefore, a second explanation inclusive of the idea of clonal expansion is that suppressor T cells directed against G² are generated in C.B-17 host mice.

Support for the second explanation comes from the demonstrations^{4,16} of G² allotype suppression in adult (SJL×BALB/c) F₁ mice that earlier were suppressed neonatally by continued exposure to anti-G² antibody. About half of the mice so treated were chronically suppressed beyond 6 months of age; the spleen cells of these mice could suppress G² production of normal (SJL×BALB/c) F₁ spleen cells both *in vivo* and *in vitro*^{5,6}. This was shown to be due to suppressor T cells which survive indefinitely as a population and apparently derive from the (SJL×BALB/c) F₁ hosts.

If the idea of autosuppressor T cells is applied to our results this suggests that the same genes which enable BALB/c T cells to suppress G² production are present in C.B-17 mice. This possibility seems reasonable since BALB/c and C.B-17 mice share the same major histocompatibility (H-2) locus and many immune functions of T cells are under the control of immune response genes that map with the H-2 locus (reviewed in ref. 17). In this

sion^{4,6}. But this consideration does not obviously apply to our results because BALB/c and C.B-17 mice do not show Ig irregularities like those in SJL mice. Therefore, the use of BALB/c congenic mice seems to provide a model autoimmune system in which to study the long term effects of constant stimulation and suppression of specific immune cells.

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Decreased resistance in some heterozygotes in H-2-linked immune response to polyoma-induced tumour

THE relationship between the major histocompatibility complex (MHC) and immune response (*Ir*) genes has been demonstrated in the case of several antigens, including some transplantation antigens¹. Among these, some tumour-associated transplantation antigens (TATA) are probably included¹. To date, however, few results are available to document this prediction. In the mouse quantitative transplantations of chemically-induced neoplasms suggest that the H-2-linked *Ir* genes of different F₁ hybrids can control their susceptibility to parent-specific tumours². This control, however, is certainly complex (that is, involving more than one gene), possibly because this sort of tumour can have different types of TATA³.

Using a better defined antigen, the X1 leukaemia-specific antigen, Sato *et al.*⁴ demonstrated that the success of transplantation immunity, induced in specifically immunised mice, is H-2 dependent. Antitumour resistance is dominantly inherited in those F₁ hybrids which have appropriate *Ir* gene(s). In the present studies, we found a clearcut H-2 dependence concerning transplantation immunity against another sort of TATA, those of a polyoma-induced tumour. Also, the recent prediction of Willims *et al.*³, that some H-2 (or *Ir* genes) combinations would lead to suppression of antitumour responsiveness is substantiated by some of our results.

of the A.SW parental strain (H-2^s) and semisyngeneic F₁ hybrids of the following combinations, obtained with congenic resistant partner strains: (A/Sn × A.SW)F₁ (H-2^a/H-2^s), (A.BY × A.SW)F₁ (H-2^b/H-2^s) and (A.CA × A.SW)F₁ (H-2ⁱ/H-2^s). Theoretically, the only traits by which all these mice differ is one half of the H-2 and closely-linked genetic material. Animals of both sexes were used and equally distributed among the groups.

The results of these transplantation assays were observed as tumour incidences (number of mice dying of the tumour/number of injected mice) and by mean survival times (MST) in days (Tables 1 and 2). They are expressed in Fig. 1 as the mean of 1/MST, taking the value of 0 for mice which rejected the tumour graft. This enables the representation of the susceptibility to the tumour, using the information from two different criteria⁶. As both types of immunisation procedures (two independent experiments having been carried out with each of them) gave superimposable results, all the data are combined in Fig. 1.

It can be seen that immunisation considerably diminished susceptibility in the A.SW and (A/Sn × A.SW)F₁ groups, whereas no protective effect was detected in (A.BY × A.SW)F₁ and (A.CA × A.SW)F₁ groups. This confirms that some F₁ hybrids can be less resistant than the parental strain to a parental strain-specific tumour⁷⁻⁹, even after immunisation.

This seems somewhat aberrant because the H-2-linked *Ir* genes generally are dominantly expressed for the highest response. Different mechanisms can be proposed for explaining the present finding: (1) A cross immunity between the TATA of SEWA and normal transplantation antigens contained in these hybrids which are not immunised against

Table 1 Susceptibility of mice challenged with 10³ viable SEWA cells intraperitoneally, after specific protection by amputation of the tumour-bearing leg (pool of two independent experiments)

Strains	H-2	Treatment	Tumour incidence	MST ± s.e. (d)
A.SW	s/s	Non-treated	9/10	47.3 ± 2.0
		Protected	1/9	71
(A.BY × A.SW)F ₁	b/s	Non-treated	2/2	34.0 ± 2.0
		Protected*	3/3	35.6 ± 2.0
(A/Sn × A.SW)F ₁	a/s	Non-treated	14/16	49.3 ± 5.9
		Protected	1/12	53.0
(A.CA × A.SW)F ₁	f/s	Non-treated	10/10	46.1 ± 1.4
		Protected	3/10	41.0 ± 6.2

*In the first experiment of protection, all mice in this group died of tumour after the amputation of the tumour-bearing leg, in spite of the fact that they were treated according to the same schedule as the mice of the other groups. This further suggests that the mice of this genome are less resistant. Results for mice in this experiment were excluded from the Table.

The SEWA line used was derived from a polyoma-induced osteosarcoma⁵. It was maintained *in vivo* in A.SW mice by intraperitoneal, consecutive passages as an ascitic tumour. Non-treated and specifically immunised animals were injected. Two modes of immunisation were used: (1) Amputation of a tumour-bearing leg, the site of subcutaneous injection of 4 × 10⁴ ascitic SEWA cells 15–25 d previously (Table 1). (2) Three weekly subcutaneous injections of 10⁶ plaque-forming units (PFU) polyoma virus (small plaque strain) (Table 2). One to three weeks after completion of immunisation, mice (three months old) received an intraperitoneal challenge of 10³ viable cells. Mice used were

the SEWA tumour could be envisaged. Such common transplantation specificities between TATA and normal allogeneic antigens have already been reported in the case of chemically-induced tumours^{8,10}. (2) Also, one example of dominantly expressed non-response of an H-2-linked *Ir* gene has been reported¹¹. Thus, allowing a good anti-TATA response the H-2^s/H-2^s chromosome of the A.SW could be recessive in the H-2^s/H-2^b and H-2^s/H-2ⁱ heterozygotes. We do not, however, favour *a priori* such an explanation because all other works reported dominance of the response and not of the non-response. The finding reported in ref. 10 seems to be most exceptional.

Table 2 Susceptibility of mice challenged with 10³ viable SEWA cells intraperitoneally, after specific protection by three weekly subcutaneous injections of 10⁶ PFU polyoma virus (pool of two independent experiments)

Strains	H-2	Treatment	Tumour incidence	MST ± s.e. (d)
A.SW	s/s	Non-treated	10/15	39.6 ± 3.4
		Protected	0/15	—
(A.BY × A.SW)F ₁	b/s	Non-treated	5/5	37.4 ± 1.2
		Protected	9/11	33.1 ± 3.0
(A/Sn × A.SW)F ₁	a/s	Non-treated	9/10	37.8 ± 2.2
		Protected	0/10	—
(A.CA × A.SW)F ₁	f/s	Non-treated	8/8	48.7 ± 0.8
		Protected	8/10	47.0 ± 2.3

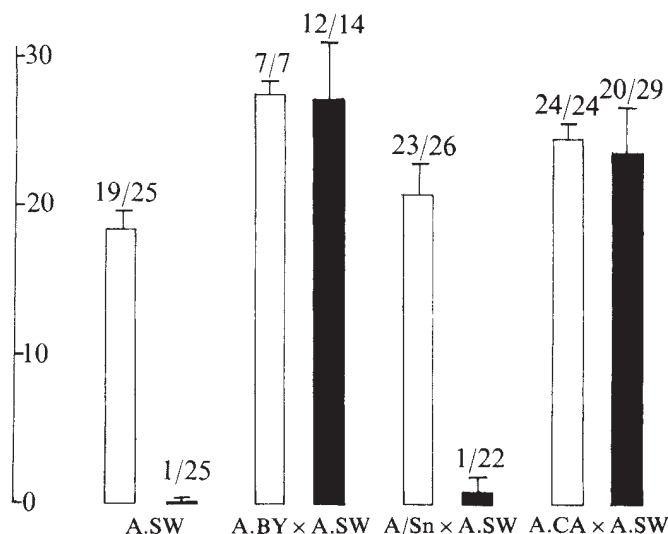


Fig. 1 Susceptibility, expressed as 1/MST (arbitrary units) of A.SW, (A.BY x A.SW)_{F1}, (A/Sn x A.SW)_{F1} and (A.CA x A.SW)_{F1} to 10³ SEWA tumour cells. Open column, non-treated mice; Solid columns, mice immunised by two different procedures; error bars are 1 s.e.; figures above bars, pooled tumour incidence of corresponding experimental groups.

(3) That $H-2^b/H-2^b$ and $H-2^s/H-2^s$ are less protected against the tumour graft than $H-2^s/H-2^s$ could be explained, on the basis of a higher response of the heterozygotes, in two ways: (i) The non-protected F_1 hybrids could have a higher anti-TATA reaction giving rise to the production of enhancing antibodies, as already discussed in another situation by Huemer⁷. Further experiments could clarify this point, by transferring the serum of non-protected immunised hybrids to test animals (ii) Recent findings led Prehn to hypothesise that a mild immune reaction, in certain cases, could enhance tumour growth instead of suppressing it¹². Although this sort of mechanism is difficult to prove in the present examples, it may explain our results.

In conclusion, and whatever the exact mechanism, the results demonstrate that one half of the $H-2$ and $H-2$ -associated genetic material may detectably influence the intensity of the anti-TATA immune rejection of a polyoma-induced tumour.

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Sprouting of synapses after partial denervation of frog cardiac ganglion

SYNAPTIC connections in the adult nervous system of several species can be quite flexible and this characteristic has been termed neuronal plasticity. For example, when lesions are made in the axonal pathways of the brains of mature rats, surviving nerve terminals can proliferate and innervate the synaptic sites vacated by the degenerating axons^{1,2}. This "sprouting" occurs in the central nervous system³⁻⁵, autonomic nervous system⁶ and peripheral nervous system⁶, and seems to be a characteristic response of nervous tissue to partial denervation. In only a few studies has sprouting been reported not to occur⁷⁻⁹. Sprouting of synapses has important implications for the maintenance of neuronal connections and the ability of the nervous system to recover from focal lesions. But several questions need to be answered. For example, are sprouted synapses functional, and if so, do they transmit normally? Is there any specificity in or control over the proliferation of new connections? What initiates and terminates the sprouting? We can now begin to answer these questions. Our studies on the parasympathetic cardiac ganglion of the frog have shown that synaptic sprouting occurs very rapidly after partial denervation. Furthermore, the number of boutons per cell remains constant throughout sprouting and is the same as the number per cell in control ganglia.

The cardiac ganglion in the frog, which lies in the thin, interatrial septum¹⁰, was dissected from the heart and kept in a shallow recording chamber in frog Ringer (112 mM NaCl, 2 mM KCl, 3 mM HEPES buffer (pH 7.2) and 5 mM CaCl₂). The calcium concentration was increased slightly to enhance synaptic transmission¹². The preganglionic (vagus) nerves were drawn into separate suction electrodes to stimulate right or left vagus nerves independently. Ganglion cells on the left branch of the septum (Fig. 1) were impaled

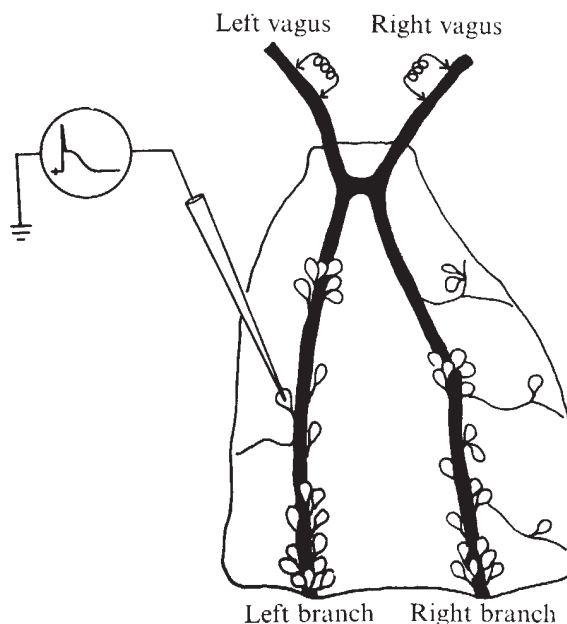


Fig. 1 Schematic drawing of the interatrial septum in the frog showing the arrangement of stimulating and recording electrodes. The cardiac ganglion cells are scattered along branches of the vagus nerves which traverse the septum. Near the entry to the heart many axons cross between left and right branches. The left and right vagus nerves to the heart were drawn into separate suction electrodes. Ganglion cells along the left vagal branch were impaled and their synaptic input from either (both) vagus nerve(s) was mapped.

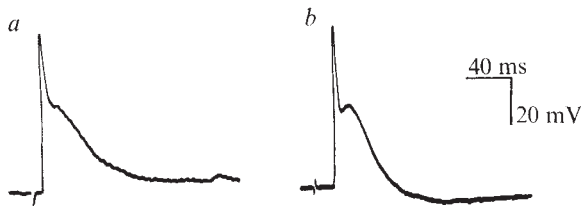


Fig. 2 Intracellular recordings of responses from ganglion cells, evoked by stimulation of the right vagus nerve in a normal ganglion (*a*) and in one in which the right vagus nerve had sprouted to innervate the entire ganglion (*b*). In both instances a large excitatory postsynaptic potential evoked an impulse and outlasted the action potential. In (*b*), the left vagus nerve had been crushed 16 d previously; the left vagus axons had degenerated and the right vagus endings had proliferated to contact all the ganglion cells.

with high resistance micropipettes and excitatory post-synaptic responses to right and left vagal stimulation were photographed. In some frogs, the left vagus nerve was exposed near its exit from the skull and crushed while the animals were anaesthetised with 0.5% tricaine methanesulphonate (Sigma). These operated animals were kept at room temperature in running tap water.

To study the sprouting of synaptic connections in the ganglion after partial denervation, we mapped the vagal innervation of cells on the left branch in normal frogs and operated frogs. In normal frogs, each ganglion cell is usually innervated by only one or sometimes two vagal fibres², and, as might be expected, neurones tend to be innervated by the ipsilateral vagus nerve. Eighty-seven per cent of the cells on the left branch had input(s) from the left vagus nerve and 48% had input(s) from the right vagus nerve (207 cells from 15 ganglia). A small proportion of these cells (34%) had inputs from both nerves. An even smaller fraction (3%) had no inputs.

When the vagal innervation of neurones on the left branch was mapped in the operated frogs, nearly all the cells received input(s) from the intact (right) vagus nerve.

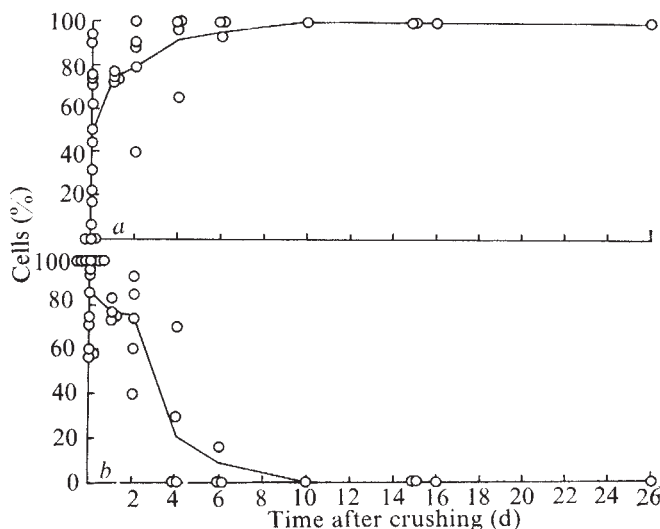


Fig. 3 Sprouting of preganglionic nerve fibres to the parasympathetic cardiac ganglion in the frog; increase in the percentage of ganglion cells innervated by the right vagus nerve after the left vagus nerve had been crushed. *a*, Percentage of neurones from the left branch of the cardiac ganglion which received synaptic input from the contralateral vagus nerve on various days after crushing the ipsilateral vagus nerve. *b*, Percentage of neurones which received synaptic input from the left vagus nerve after the operation. Day zero shows data from 15 unoperated ganglia. Each symbol represents data from one ganglion (20–30 cells impaled in each ganglion) and a solid line has been drawn through the weighted means. Percentages were calculated on the basis of the total number of cells which received any synaptic input.

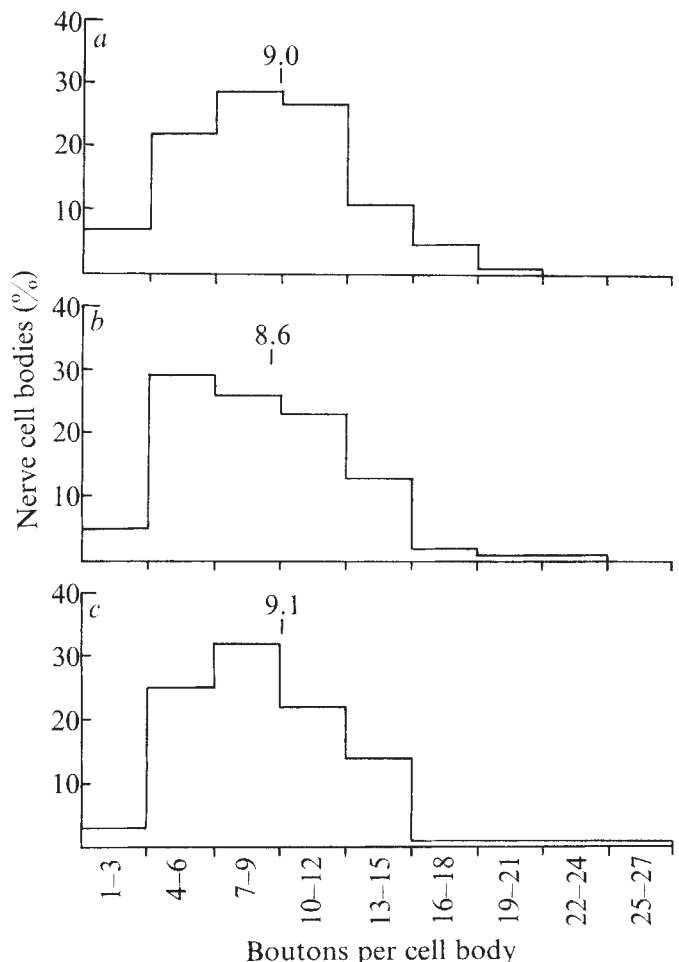
For example, 10–26 d after the left vagus nerve was crushed, 100% of cells with synaptic inputs was innervated by the right vagus nerve (compare 48% for unoperated frogs). Six per cent had no inputs; this latter figure is similar to that in normal animals and presumably due to damage caused during dissection. Because there was no visible loss of ganglion cells after the operation, we interpret this increased percentage of contralateral innervation as due to a proliferation, or sprouting, of the surviving right vagus nerve.

The “sprouted synapses” are functional, and as far as we could detect, transmit normally (Fig. 2). No abnormal synaptic responses were evoked during or after sprouting. Experiments are in progress to examine synaptic transmission in greater detail to determine whether we can resolve any peculiarities of sprouting synapses.

The time course of sprouting was examined by mapping vagal innervation of different ganglia at several intervals after the operation. Figure 3 shows that as soon as 24 h after crushing of the left vagus nerve, there was a small but significant ($P < 0.01$) increase in innervation by the right vagus nerve. The maximum extent of sprouting was reached about 8 days after the operation; there was no further increase in the proportion of cells innervated by the right vagus nerve.

To study the morphological basis of this phenomenon

Fig. 4 Histograms of numbers of synapses per cell body before (*c*), during (*b*), and after (*a*) sprouting of preganglionic inputs. Synaptic boutons were counted in whole mounts of ganglia stained with zinc iodide. In *c*, boutons from 73 neurones on the left branches in nine unoperated ganglia were counted. In *b*, boutons were counted from 112 ganglion cells (left branch) from animals in which the left vagus nerve had been crushed 1–8 d previously. In *a*, boutons were counted from 120 neurones (left branch) from animals 9–26 d after the operation. Numbers above each histogram are the mean values.



and determine whether we could correlate the changes in innervation as determined physiologically with any structural changes, we stained synaptic boutons in whole-mount preparations with the zinc-iodide method^{10,11} for light microscopy. Although the basis of the zinc iodide method is not well understood, we assume that there is no difference in the ability of sprouted synapses and normal synapses to take up the stain. In normal frogs, the average number of synaptic boutons per cell on the left branch was 9.1 ± 4.3 . When boutons were counted in ganglia after the left vagus nerve had been crushed, and sprouting of the right vagus nerve was complete, there was no change (9.0 ± 3.9 boutons per cell). Boutons were counted at several intervals after crushing of the left vagus nerve. Figure 4 summarises the measurements and shows that there was no change in the number of boutons per cell during sprouting (1–8 d after the operation) or after its completion (9–26 d after the operation).

These findings indicate that surviving vagal axons respond rapidly to partial denervation of the ganglion by sprouting and form new functional synapses. Furthermore, the morphological studies show that sprouting is a well controlled phenomenon. Thus, the proliferation of synapses does not change the number of boutons from that found on normal cells. These findings are consistent with the hypothesis that sprouting synapses grow to vacant synaptic sites² and that each cell has a fixed number of these sites⁷.

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Molecular differentiation in the preimplantation mouse embryo

THE primary observable event of differentiation in the mammalian embryo is the formation of the trophoblast and the inner cell mass (ICM). Differentiation first becomes evident in the mouse embryo during early blastulation and is clear and apparently irreversible by the 60-cell blastocyst stage. The 45 or so trophoblast cells surround a blastocoelic cavity containing the ICM, they pump fluid, are phagocytic, are interconnected through junctional complexes and can elicit an implantation response in the uterus¹. The trophoblast gives rise to the ectoplacental trophoblast, primary and secondary trophoblastic giant cells and probably also to extra-embryonic ectoderm^{1,2}. The cells of the ICM are less evidently differentiated, being rounded, sticky cells somewhat like cleavage stage blastomeres, but having lost the capacity to form trophoblast^{3,4}. The ICM gives rise to embryonic ectoderm, and to both extra-embryonic and embryonic endoderm^{1,2,5}. Although

the fully differentiated cells of the blastocyst are distinguishable by structural and functional criteria, adequate markers of differentiation are not available for the morula stage, when the determinative events leading to differentiation are presumed to occur. Thus, attempts to detect blastomeres with a commitment to one or the other differentiated cell type have relied for an index of commitment on the subsequent behaviour of genetically or physically marked blastomeres combined in various spatial relations with other distinguishable blastomeres. This experimental approach is open to criticism. Frequently, many cell divisions must elapse before suitable genetic markers can be detected with sufficient sensitivity, and the procedures involve artificial manipulation and therefore possibly modification of blastomeres to test their potency^{2,6}. The detection of a cell marker which is produced *in situ* in the earliest stages of differentiation, and one which is detectable by highly sensitive techniques would circumvent both of these problems. We describe here the detection of tissue specific markers which may prove to fulfil these criteria.

Mouse blastocysts were flushed from the uteri of super-ovulated mice of an inbred strain carrying the *a^c* gene (extreme non-agouti) which had been mated to *a^b/a^b* males 3.5 d previously (detection of vaginal plug indicated day 0). All blastocysts had recently expanded, with zona pellucida intact and with no evidence of trophoblastic giant cell formation. Blastocysts from several females were pooled for each experiment and then divided into three groups: the first group was left intact, the second group was used for microdissection of ICMs and the third for microdissection of trophoblastic vesicles¹. Dissection of the trophoblastic vesicles involves loss of the polar trophoblast overlying the ICM. Occasionally both trophoblastic vesicles and ICMs were recovered from the same blastocysts. Microdissected fragments were incubated in medium PB1+10% foetal calf serum for 2–3 h, and then checked to determine that only cells in the trophoblastic preparation and not those in the ICM preparation were accumulating fluid. This procedure served to test for any major cross contamination of cell type. The embryos and embryonic fragments were then washed in protein-free medium 16 (ref. 7), and transferred to 100 μ l of medium 16 containing 10 μ l of ³⁵S-L-methionine (New England Nuclear, specific activity 200 Ci mmol⁻¹). After incubation for 4 h at 37 °C in a moist atmosphere of 5% CO₂ in air, the blastocysts and fragments were collected into 25 μ l of lysis buffer containing urea, 2-mercaptoethanol, Ampholines (LKB, pH 5–7 and 3–10) and non-ionic detergent Nonidet P-40.

It was confirmed that the radiolabelled amino acid had been incorporated into proteins by measuring trichloroacetic acid-precipitable counts of aliquots of each sample⁸. Microdissection of embryos did not seem in most experiments to reduce the ability of fragments to incorporate label, when allowance was made for the loss of approximately one third of embryonic tissue during dissection. The relative rate of protein synthesis was comparable with that previously observed in intact blastocysts at the same stage of development⁹. In each of six experiments, a minimum of 8–10 microdissected fragments of each tissue was used.

Labelled proteins were separated by two-dimensional polyacrylamide gel electrophoresis⁸. After separation of proteins by isoelectric focusing on cylindrical gels, proteins were electrophoresed into exponential (8–15%) gradient, sodium dodecyl sulphate (SDS)–polyacrylamide slab gels^{8,9}. After electrophoresis, the slab gels were infiltrated with the scintillant PPO¹¹, dried under vacuum, placed under X-ray film (Kodak Royal-X-Omat, RP/R54) and stored at –70 °C for 6–10 weeks before developing. Each spot detected on the autoradiographs represented one polypeptide into which label had been incorporated.

Representative two-dimensional protein patterns of intact blastocysts (*a*), cells of the ICM (*b*) and cells of the trophoblast (*c*) are shown in Fig. 1. Patterns were compared for protein spots common to all three preparations (intact embryo, Fig. 1*a*), spots limited to the ICM and intact embryo (indicated by arrows, Fig. 1*b*) and spots limited to the trophoblast and intact embryo (indicated by solid triangles, Fig. 1*c*). No spots were found exclusively in intact embryos. Most polypeptide spots were common to all three types of preparations, as might be expected since they presumably represent proteins required for general cell function. Several 'tissue-specific' spots, however, were seen consistently. The term 'tissue-specific' spots describes those polypeptides which were either not detectable or only very weakly so in one dissected tissue but clearly present in the other. An occasional faint trace of some of the stronger trophoblast-specific spots was detected on some gels of the ICM (Fig. 1*b*), and vice versa (Fig. 1*c*). Such spots might represent a genuine observation but may alternatively be due to cross contamination by a few cells, made visible by the sensitivity of the technique.

Apart from many apparent quantitative differences between individual spots in the patterns obtained from dissected ICM and trophoblast, ICM tissue yielded at least eight consistent tissue-specific spots (arrows, Fig. 1*b*), while trophoblast tissue yielded a number of obvious, major

'tissue-specific' spots (Fig. 1*c*) of which three spots constituted a relatively substantial fraction of the total incorporated material in the tissue (Fig. 1*c*). In addition, at least three other spots were found in ICM dissections but occurred in either of two different positions relative to all other spots in different experiments (asterisks, left of *b* and *c*). One possible explanation for this observation is that at least three polypeptides differ in the tissues from different dissections. Such a difference could result from genetic heterogeneity in the a^e strain blastocyst or perhaps from the chemical modification of polypeptides, such as the addition or deletion of a carbohydrate group. The chemical modification of polypeptides may be a normal feature of development and could be related to the age of the blastocysts or the position of cells within the embryo. Nine other spots (not marked) were detected in the trophoblast only or in the ICM only but were not consistently observed in the microdissections. These polypeptides were only weakly radioactive and may be more clearly defined with prolonged exposures or with preflashing of the X-ray film¹¹.

The failure to detect polypeptide spots unique to intact blastocysts implies that the polar trophoblast overlying the ICM probably does not synthesise a unique set of proteins. One spot which was, however, very weak in the gels of intact embryos (Fig. 1*a*) was relatively intense in gels of ICM (arrow 1, Fig. 1*b*) and absent from the gels of the trophoblast (Fig. 1*c*). This observation could be explained by an ICM-trophoblast interaction, in which quantitative rather than qualitative regulation of protein synthesis was involved. In this case interactions between ICM and trophoblast may have functioned to limit rather than induce the synthesis of a particular polypeptide.

These results have identified molecular correlates of the earlier visible events of differentiation in the mouse. The existence of differences at the molecular level is not surprising in view of the overt differentiation at the cellular level¹². Indeed, the fact that only a relatively minor proportion of the spots identified are 'tissue-specific' might be considered surprising. But we are not detecting all newly synthesised proteins with this technique, since the limits between which both isoelectric focusing and gradient electrophoresis function will exclude many proteins. Other polypeptides will be only weakly radioactive and will require longer exposure to preflashed X-ray film for detection. In addition, some autoradiographic spots may not necessarily represent the direct expression of new gene products but rather, as a normal feature of development,

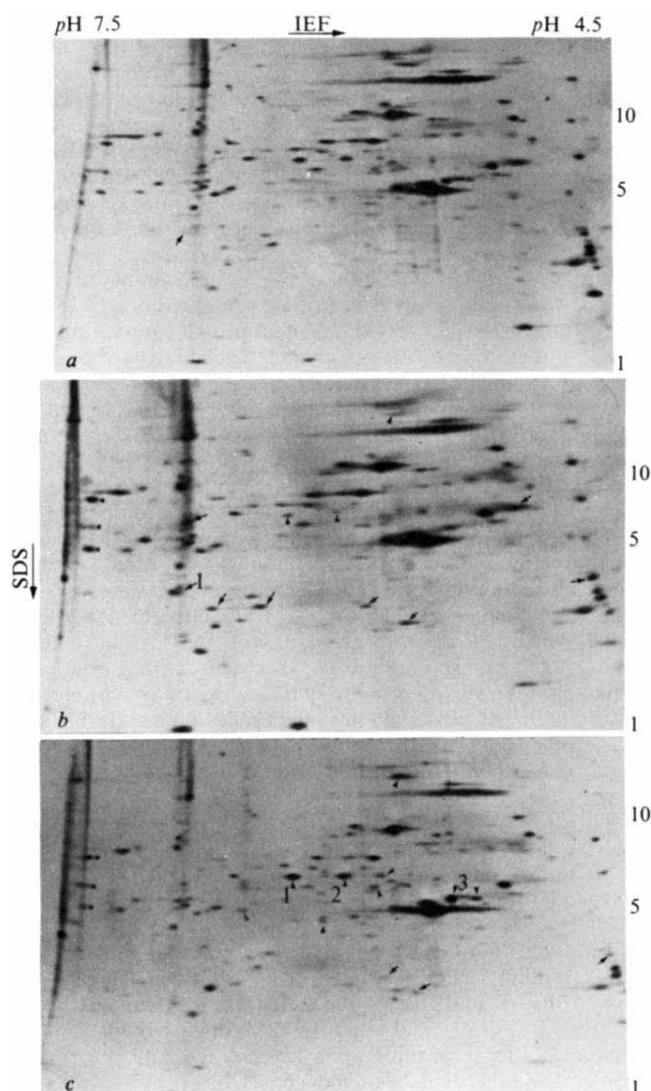


Fig. 1 *a*, Separation of protein labelled with ^{35}S -L-methionine from intact, 3.5-d a^e blastocysts by high resolution, two-dimensional gel electrophoresis. In the first dimension, proteins were separated according to charge by isoelectric focusing (IEF) on 135-mm cylindrical gels. In the second dimension, proteins were separated according to molecular weight on sodium dodecyl sulphate (SDS), exponential-gradient (8–15% acrylamide), polyacrylamide slab gels. For the first dimension, the pH range was determined by cutting a one-dimensional gel (after isoelectric focusing) into 3-mm pieces, mashing the pieces in degassed, distilled water (2 ml), shaking the mixture for approximately 1 h (in tightly sealed vials) and then measuring the pH of the mixtures. Approximate molecular weights ($\times 10^{-3}$) are given on the far right and were determined from gels calibrated for molecular weight as before⁹. The arrow points to a polypeptide which was absent from trophoblast preparations, relatively intense on ICM preparations (arrow, 1, Fig. 1*b*) but only weakly radioactive in the intact embryo. *b*, Representative autoradiograph of protein labelled with ^{35}S -L-methionine from ICM dissection. Spots limited to this tissue are indicated by arrows. Faint traces of major trophoblast spots are indicated by solid triangles. Approximate molecular weights ($\times 10^{-3}$) are given on the far right. *c*, Representative autoradiograph of protein labelled with ^{35}S -L-methionine derived from trophoblast dissection. Spots limited to this tissue are indicated by solid triangles with major spots indicated by triangles 1–3. Faint traces of major ICM spots are marked by arrows. Approximate molecular weights ($\times 10^{-3}$) are given on the far right.

the chemical or enzymatic modification of proteins, with a resulting alteration in spot position. Further study is required to define those spots representing new gene products and those spots representing polypeptides which have been modified after translation.

With the above caveats in mind, the detection of the differences described here presents a powerful approach for the analysis of progressively earlier stages of mammalian development to determine the time at which the 'tissue-specific' proteins first appear and the spatial distribution of the cells producing them. With such an approach using intact, non-manipulated and unmarked embryos, it may prove possible to test directly the hypothesis that cell position determines selective gene expression and thus differentiation in preimplantation morulae¹³⁻¹⁵.

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Increased cyclic nucleotide phosphodiesterase activity in leukaemic lymphocytes

THE intracellular concentration of cyclic AMP influences cellular proliferation and maturation in both normal and malignant tissue. For example, the concentration of cyclic AMP—changes during the cell cycle being lowest during mitosis¹—is inversely related to the rate of cell growth², and is elevated in contact-inhibited cell populations^{3,4}. In studies of malignant tissue, the concentration of cyclic AMP is lower in cells transformed *in vitro* by oncogenic viruses than in untransformed cells³⁻⁵, and is lower in certain tumours grown *in vivo* than in the corresponding normal tissue^{6,7}. Furthermore, in malignant cells, exogenous cyclic AMP or agents that increase the intracellular concentration of this cyclic nucleotide decrease the rate of growth⁸ and induce morphological and biochemical differentiation^{9,10}.

The role of cyclic GMP in cell growth is less clear although it has been demonstrated that its concentration increases in lymphocytes responding to plant mitogens¹¹ and decreases in fibroblasts transformed by oncogenic viruses¹².

A decreased concentration of cyclic AMP in malignant cells may be caused by a reduction in the activity of the

enzyme catalysing its synthesis (adenylate cyclase) or an increase in the activity of the enzyme catalysing its hydrolysis (phosphodiesterase). There is evidence that both these enzymes are altered in certain transformed cells. For example, BHK cells¹³ and astrocytoma cells¹⁴ transformed by oncogenic viruses have lower activities of adenylate cyclase than do untransformed cells. Other types of transformed cells and certain solid tumours did not, however, always exhibit lower activities of adenylate cyclase (for reviews see refs 15 and 16).

Most studies of phosphodiesterase showed that enzyme activity was lower in malignant cells than in normal cells (for review see ref. 17). Other studies showed no change in phosphodiesterase activity in malignant cells¹⁴, and still others¹⁸ have shown that although hepatomas have a decreased total activity of phosphodiesterase, the activity of the low K_m form of the enzyme was increased.

We now report that certain leukaemic lymphocytes have markedly increased activities of both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase compared with those of normal lymphocytes. This increased capacity to hydrolyse the cyclic nucleotides may provide an explanation for the abnormal growth of these cells.

Female mice (20-25 g, Jackson Laboratories, Bar Harbor, Maine) were given free access to food and water. Lymphocytic leukaemia cell lines, L5178Y and L1210 (Little, Cambridge, Massachusetts) were grown in the ascites fluid of the peritoneal cavity of C₃D₂F₁ mice. Activities of cyclic AMP

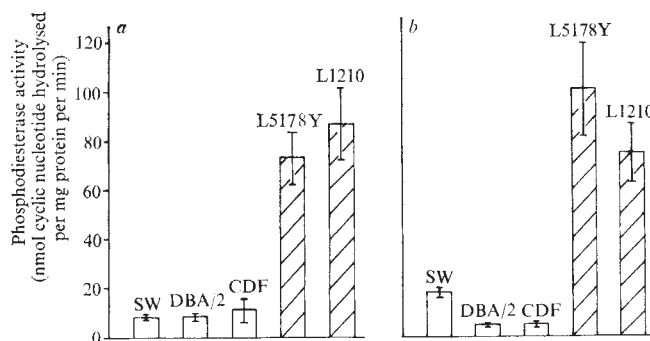


Fig. 1 Cyclic nucleotide phosphodiesterase activity of normal and leukaemic lymphocytes. Normal lymphocytes (open bars) were prepared from the lymph nodes of three strains of mice: DBA/2, the strain in which the tumours originated; C₃D₂F₁ (CDF), the strain that serves as host for the tumour; and Swiss Webster (SW), a widely used inbred strain. Lymph nodes were minced in Krebs-Henseleit buffer, pH 8.0. Cells were freed from the stroma in a Dual No. 24 homogeniser using a loose fitting Teflon pestle. Cell suspensions were purified by passage through a plastic column fitted with 40×7 mm of loosely-packed siliconised glass wool. Cells were washed twice in buffer and collected by centrifuging at 480g for 10 min. Cells were sonicated for 15 s at 100 W in 50 mM glycylglycine buffer, pH 8.0. Leukaemic lymphocytes, L5178Y and L1210 (hatched bars), were collected 6 d after transplantation into CDF mice by injecting 2 ml Krebs-Henseleit buffer intraperitoneally and aspirating the cell suspension. Cells were prepared in the same manner as were the normal lymphocytes. Using these techniques, both preparations contained more than 95% lymphocytes; 99% of the cells were viable as evidenced by their ability to exclude Trypan blue. Cells were sonicated for 15 s, at 100 W, which was long enough to disrupt more than 90% of the cells without appreciable loss of phosphodiesterase activity. Cyclic nucleotide phosphodiesterase was measured in the whole sonicate using either cyclic AMP (250 μM) or cyclic GMP (100 μM) as substrate. Each tube contained a 50 mM glycylglycine buffer (pH 8.0) containing 0.1 mM CaCl₂ and an enzyme cycling system described in detail elsewhere^{19,20}. Protein was measured by the method of Lowry *et al.*²¹. Activity of both cyclic nucleotide phosphodiesterases (on a protein basis) was significantly greater in the leukaemic lymphocytes than in the normal lymphocytes ($P < 0.005$). When the activity was based on the number of cells, the difference in activity between the normal and leukaemic cells was two to three times greater than when the activity was based on protein. Bars represent mean ± s.e. of at least five enzyme preparations. *a*, Cyclic AMP phosphodiesterase; *b*, Cyclic GMP phosphodiesterase.

Table 1 Kinetic analysis of cyclic nucleotide phosphodiesterases of normal and leukaemic lymphocytes

Cell Source	Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
	$V_{\max}$	K_m (μM)	$V_{\max}$	K_m (μM)
Normal				
Swiss Webster	11 $\pm$ 2	85 $\pm$ 12	45 $\pm$ 5	38 $\pm$ 5
DBA/2	11 $\pm$ 1	67 $\pm$ 7	7.6 $\pm$ 1.6	40 $\pm$ 4
C ₃ D ₂ F ₁	22 $\pm$ 6	102 $\pm$ 14	9.6 $\pm$ 1.2	32 $\pm$ 5
Leukaemic				
L1210	224 $\pm$ 20	159 $\pm$ 29	124 $\pm$ 28	60 $\pm$ 7
L5178Y	141 $\pm$ 31	113 $\pm$ 18	225 $\pm$ 48	52 $\pm$ 9

Normal and leukaemic lymphocytes were prepared as in Fig. 1. Cells were sonicated and centrifuged at 100,000g for 60 min. The activity of cyclic AMP and cyclic GMP phosphodiesterase was measured in the supernatant fraction. $V_{\max}$ and K_m were calculated according to the procedure of Hofstee³⁰ at substrate concentrations from 15–250 μM cyclic AMP and 12.5–100 μM cyclic GMP. $V_{\max}$ for both cyclic nucleotide phosphodiesterases was significantly greater in the leukaemic cell preparations than in any of the normal cell preparations ($P < 0.005$ for all preparations except for Swiss Webster cyclic GMP phosphodiesterase where $P < 0.01$). Each value represents the mean $\pm$ s.e. for three to five enzyme preparations. Enzyme activity is expressed as nmol cyclic nucleotide hydrolysed per mg protein per min.

phosphodiesterase¹⁹ and cyclic GMP phosphodiesterase²⁰ were determined as described previously. The rate of hydrolysis of the cyclic nucleotides in these assays was linear with respect to the time of incubation and concentration of tissue. The effect of tissue on the standard curves was determined and, if necessary, appropriate corrections were made. All experiments were designed to study the enzymes from freshly prepared normal and leukaemic lymphocyte preparations on the same day in identical assay conditions.

Figure 1 compares the specific activities of cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase in whole sonicates of leukaemic and normal lymphocytes. As can be seen, the phosphodiesterase activity in the leukaemic lymphocytes was 5–10-fold greater than that of any of the other three normal lymphocyte preparations studied. To determine if this increase in phosphodiesterase activity of the leukaemic cell preparations was confined to a specific subcellular element in these cells, we studied the phosphodiesterase activity in various subcellular fractions as well as in the 100,000g supernatant fluid. Leukaemic cells exhibited a greater phosphodiesterase activity in all subcellular fractions studied.

A kinetic analysis of the 100,000g supernatant fraction of the lymphocyte preparations is shown in Table 1. The activity of phosphodiesterase was determined at substrate concentrations from 1 to 250 μM for cyclic AMP and from 0.8 to 100 μM for cyclic GMP. At all substrate concentrations, the phosphodiesterase from leukaemic cells hydrolysed cyclic AMP and cyclic GMP at a greater rate than did the phosphodiesterase of normal lymphocytes. The calculated maximum velocities ($V_{\max}$) for both the cyclic nucleotide phosphodiesterases from either the L1210 or L5178Y leukaemic lymphocytes was markedly greater than the $V_{\max}$ of any normal lymphocyte preparation.

This increased reaction velocity could not be attributed

to an increased apparent affinity of the enzyme from the leukaemic cell for its substrate since preparations from normal lymphocytes showed similar or even lower apparent Michaelis constants (K_m values) than did the preparations from the leukaemic cell lines. At concentrations of cyclic AMP from 15 to 250 μM or at concentrations of cyclic GMP from 12.5 to 100 μM , each phosphodiesterase exhibited a single K_m value. At lower substrate concentrations, complex kinetic behaviour made accurate assessment of other K_m values impractical. The complex kinetics observed may indicate the presence of a mixture of isoenzymes in these cells as has been found in most tissues^{21,22} including single cell lines²³.

Several possibilities may account for the observed increase in phosphodiesterase activity of leukaemic lymphocytes. For example, the difference may be attributable to the different environments in which the cells were grown, as the leukaemic cells were in suspension in the ascites fluid of the peritoneal cavity, whereas the normal lymphocytes were in a solid tissue. To examine this possibility we injected leukaemic lymphocytes subcutaneously into CDF mice. Six days later the solid nodule was dissected, and the cells were freed from stroma by the procedure described for the preparation of normal lymphocytes (see Fig. 1). The phosphodiesterase activity of these cells (73 $\pm$ 4 nmol cyclic AMP and 65 $\pm$ 5 nmol cyclic GMP hydrolysed per mg protein per min) was not significantly different from that of leukaemic cells grown for 6 d in the ascites fluid of the peritoneal cavity of another group of CDF mice (86 $\pm$ 5 nmol cyclic AMP and 59 $\pm$ 3 nmol cyclic GMP hydrolysed per mg protein per min) indicating that the greater phosphodiesterase activity in leukaemic lymphocytes was probably not caused by the physical environment of the cells.

We next examined the possibility that the increased activity of phosphodiesterase in leukaemic cells could be attributed to the presence in these cells of a heat-stable, calcium-dependent, protein activator of phosphodiesterase which has been found previously in almost all other tissues^{24,25}. We assayed for the presence of this activator in leukaemic cells according to the method of Cheung²⁵. These cells did contain an activator of phosphodiesterase as evidenced by its ability to increase the activity of phosphodiesterase from mouse cerebrum fivefold (Table 2). It had no effect, however, on the phosphodiesterase from leukaemic lymphocytes. This suggested either that the phosphodiesterase from leukaemic lymphocytes could not be activated or that it was already maximally activated. To examine this latter possibility we measured the phosphodiesterase activity in the presence of the calcium chelator, EGTA. As can be seen (Table 2) EGTA produced a 75% inhibition of phosphodiesterase in brain (suggesting that this enzyme was already partially activated) but had no

Table 2 Effects of phosphodiesterase activator from leukaemic lymphocytes on the cyclic AMP phosphodiesterase activity of cerebrum and leukaemic lymphocytes

Phosphodiesterase source	Cyclic AMP phosphodiesterase		
	Control	Activator	EGTA
Cerebrum	56 $\pm$ 4	217 $\pm$ 11*	16 $\pm$ 1*
Leukaemic lymphocytes	61 $\pm$ 3	59 $\pm$ 6	68 $\pm$ 3

Activator of phosphodiesterase was prepared from leukaemic lymphocytes according to Cheung²⁶. Activity of phosphodiesterase was determined in whole sonicates of cerebrum of DBA/2 mice and L1210 leukaemic lymphocytes in the presence of 0.1 mM Ca²⁺ and 3 mM Mg²⁺ in the absence and presence of activator (25 μl) or EGTA (1 mM) using 250 μM cyclic AMP as substrate. Activity is expressed as nmol cyclic AMP hydrolysed per mg protein per min. Each value represents the mean of four determinations $\pm$ s. e.

* $P < 0.001$ compared with control values.

effect on the phosphodiesterase of the leukaemic lymphocytes. In addition, when portions of enzyme preparations from normal lymphocytes were added to the enzyme from leukaemic cells, the phosphodiesterase activity was additive, suggesting that no inhibitor of phosphodiesterase was present in the normal cells. Thus, the greater activity of phosphodiesterase in leukaemic lymphocytes apparently was not caused by the presence of a phosphodiesterase more responsive to the endogenous activator or by a phosphodiesterase in a highly activated state, but rather was attributable to a greater quantity of the enzyme in the leukaemic cells.

Based on the present findings and on the evidence that cyclic AMP inhibits the rate of growth of these leukaemic lymphocytes^{26,27}, one may speculate that the abnormal proliferation of these cells may be explained by an increase in the activity of the enzyme that catalyses the hydrolysis of cyclic AMP. The significance and interpretation of the high activity of cyclic GMP phosphodiesterase in leukaemic lymphocytes must await studies on the role of cyclic GMP in these cells. Further, since cyclic AMP phosphodiesterase exists in multiple forms²¹⁻²³, which can be selectively inhibited by drugs²⁸, it may be possible to inhibit specifically the predominant forms of the enzyme in these malignant cells. This might cause an elevation in the concentration of cyclic AMP, and consequently a reduction in the growth rate of the abnormal cells, leaving normal cells unaltered.

These studies on the multiple forms of phosphodiesterase as well as studies which are designed to show whether other qualitative differences exist between the phosphodiesterases of normal and leukaemic lymphocytes are currently in progress. It would be particularly instructive to determine, for example, if the increased activity is attributable to a single enzyme which hydrolyses both cyclic AMP and cyclic GMP or to a specific increase in an enzyme form with specificity for either of the two cyclic nucleotides.

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Spectrophotometric evidence for the presence of phytochrome in the envelope membranes of barley etioplasts

PHYTOCHROME is a chromoprotein photoreceptor, existing in two photoconvertible forms (Pr and Pfr), and is responsible for various metabolic and developmental responses of higher plants to light¹. The location of phytochrome in plant cells and its mechanism of action are matters of debate. Many physiological responses to light have been adduced as evidence for phytochrome either being a component of certain cellular membranes, or acting on them, bringing about rapid changes in cell metabolism which lead to developmental changes². There have been claims that phytochrome, in the Pfr form only, binds specifically to as yet uncharacterised membranous fractions³⁻⁶ and the data have formed the basis for allosteric models of the action of phytochrome³⁻⁷. Further progress requires a demonstration of the precise location of phytochrome with relation to a well characterised membrane where it is known to direct a process *in vitro*. We demonstrated^{8,9} the specific association of a proportion of total cellular phytochrome with etioplasts isolated from 6-d-old etiolated barley leaves. Etioplast phytochrome, as a result of its interconversions, was shown to regulate, *in vitro*, the efflux of endogenous etioplast gibberellins across the envelope membranes into the surrounding medium. Confirmatory results have been obtained with etiolated wheat leaves^{10,11}. Although phytochrome was detected spectrophotometrically in the purified etioplast preparations from barley, no data were obtained

Fig. 1 Protocol for the separation of etioplast envelopes. Procedure as in ref. 10 modified by the use of 25 mM MOPS buffer (described in Table 1) as the isolation medium. The period of lysis at 0 °C was extended to 15 min.

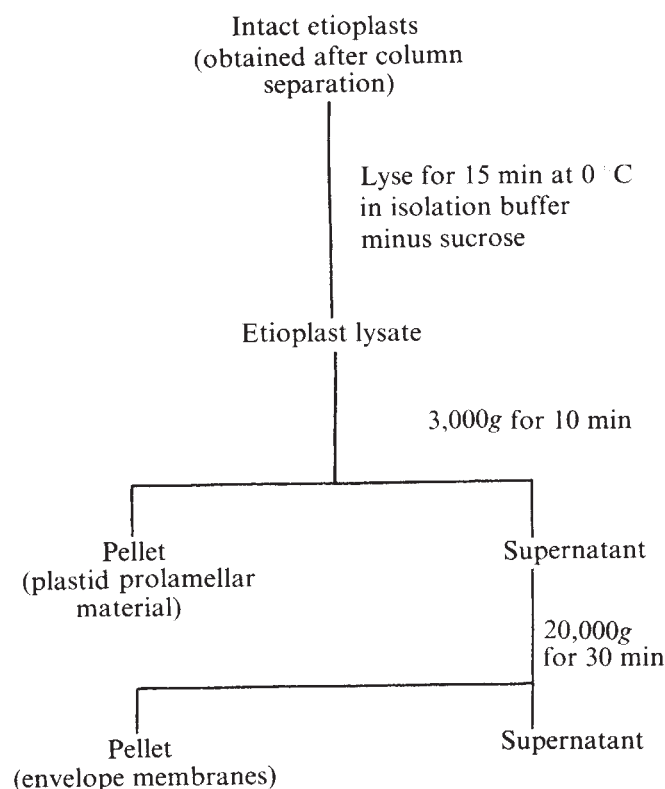


Table 1 Measurement of protein and phytochrome content at stages during isolation of etioplasts

Fraction	Protein per aliquot (mg)	$\Delta (\Delta A)$ 660/730 nm	$\Delta (\Delta A)$ per mg protein	Mean $\Delta (\Delta A)$ per mg protein	% Total phytochrome
(1) Crude homogenate	2.450	0.0100	0.00408	0.00447	100.00
	0.970	0.0040	0.00410		
	2.025	0.0100	0.00490		
	0.810	0.0038	0.00470		
	0.910	0.0042	0.00460		
(2) Crude plastid pellet	2.160	0.0028	0.00129	0.00140	1.48
	1.630	0.0022	0.00130		
	1.920	0.0025	0.00130		
	0.810	0.0015	0.00180		
	1.080	0.0014	0.00129		
(3) Supernatant	4.500	0.0205	0.00455	0.00450	89.45
	0.450	0.0020	0.00440		
	2.250	0.0105	0.00466		
	0.910	0.0040	0.00440		
(4) Washed plastid pellet	0.960	0.0024	0.0025	0.00210	1.32
	0.864	0.0018	0.0021		
	0.861	0.0022	0.0026		
	0.810	0.0015	0.0018		
	1.720	0.0030	0.0017		
(5) Supernatant	1.260	0.0000	0.0000	0.00000	0.00
	0.507	0.0000	0.0000		
	3.330	0.0000	0.0000		
	3.570	0.0000	0.0000		
	2.880	0.0000	0.0000		
(6) Purified etioplasts	0.720	0.0009	0.0013	0.00140	0.63
	0.390	0.0005	0.0013		
	1.590	0.0020	0.0013		
	1.800	0.0017	0.0009		
	1.320	0.0027	0.0021		

Etioplasts were isolated in 25 mM *N*-morpholino-3-propanesulphonic acid (MOPS) buffer containing 3 mM EDTA (disodium salt), 300 mM sucrose, 14 mM 2-mercaptoethanol and adjusted to a final pH of 7.5. Aliquots of (1) the crude homogenate, (2) the first crude plastid pellet, and (3) supernatant after a 1-min spin at 6,000g, (4) the washed plastid pellet and supernatant (5), after a second similar centrifugation, and (6) the final purified etioplasts, were taken for analysis. Samples of various volume were made up to 3 ml for dual wavelength measurement in a Perkin Elmer 156 digital double wavelength spectrophotometer. Measurement was made over a 1-cm path length using calcium carbonate as a light scattering agent. Actinic radiations lasting 30 s were of 660 nm and 730 nm. Measuring beams were also of these wavelengths.

for the suborganellar location of the photoreceptor or for the amount present in the etioplasts. We report here that although only a small proportion of the total phytochrome is associated with etioplasts, it seems to be concentrated in the envelope membranes. These data support the view that phytochrome is located in specific cellular membranes where it regulates transmembrane transport¹².

Etioplasts were isolated and purified by a modification^{8,9} of the Sephadex column technique developed by Wellburn¹³. As we have described previously^{8,9}, this method yields etioplasts which are intact and very pure as judged by

electron microscopic and biochemical criteria. Samples for protein¹⁴ and phytochrome^{8,9} measurements were taken at each separation stage.

The data from five separate preparations are presented in Table 1, showing high reproducibility. The amount of phytochrome per mg protein of the purified etioplasts was between a quarter and a third that of the crude homogenate. According to Pratt and Coleman¹⁵, however, a large proportion of the phytochrome of etiolated tissues is in a free, soluble state in the cytoplasm and not associated with specific structures. Approximately 90% of the total homogenate phytochrome remained in the supernatant after the first centrifugation, although unknown amounts may have been associated with organelles or plastid fragments of a lower pelletability than intact etioplasts. Table 1 shows that the purified etioplasts accounted for about 2% of the homogenate protein and 0.6% of the total homogenate phytochrome. Although the actual proportion of phytochrome associated with the etioplasts is small, the number of possible phenomena which it may control there makes it far from insignificant¹.

To determine the suborganellar location of etioplast phytochrome, purified etioplasts were fractionated into envelope, prolamellar and supernatant fractions according to the method of Mackender and Leech¹⁶ modified as shown in Fig. 1. The prolamellar and envelope fractions contained the expected components revealed by electron microscopy, although biochemical characterisation has not been carried out. Measurements of protein and phytochrome in each fraction for two experiments are presented in Table 2. In both cases, although the protein contents of the 3,000g pellet (that is, the prolamellar fraction) and the 20,000g supernatant were roughly twice that of the 20,000g pellet

Table 2 Measurement of phytochrome after the fractionation of etioplasts

Fraction	Protein (mg)	$\Delta (\Delta A)$ 660/730 nm	$\Delta (\Delta A)$ per mg protein	Mean $\Delta (\Delta A)$ per mg protein
Intact etioplasts	1.590	0.00200	0.00125	0.00167
	1.320	0.00270	0.00210	
3,000g pellet	1.115	0.00000	0.00000	0.00000
	1.115	0.00000	0.00000	
3,000g supernatant	1.080	0.00215	0.00199	0.00199
	1.000	0.00200	0.00200	
20,000g pellet	0.500	0.00120	0.00240	0.00241
	0.660	0.00160	0.00242	
20,000g supernatant	0.900	0.00000	0.00000	0.00000
	1.000	0.00000	0.00000	

Dual wavelength spectrophotometry was carried out on aliquots of samples made up to 3 ml.

(that is, the envelope fraction), phytochrome was only detectable in the latter. Approximately 77% of the initial etioplast phytochrome was recovered in the 20,000g pellet which in terms of protein content constitutes a 1.44-fold enrichment of phytochrome. The remaining phytochrome was lost or rendered undetectable.

Although definitive proof will depend on rigorous chemical and biochemical characterisation of the subetioplast fractions, these data strongly suggest that all etioplast phytochrome is located in the etioplast envelope. It seems unlikely that its presence there results from nonspecific adsorption, since our earlier work has shown that phytochrome exerts rapid control over transport of gibberellins across the etioplast envelope *in vitro*^{8,9}. The pelletable phytochrome investigated by many other workers⁷ seems to bind to membranous material only in the Pfr form; etioplast phytochrome, on the other hand, exists as Pr in or on the etioplast envelope. The characteristics of etioplast phytochrome, therefore, are not easily reconcilable with the concept of phytochrome existing as soluble Pr in the cytoplasm and moving to specific binding sites on membranes only when photoconverted to Pfr, as proposed in the allosteric models of phytochrome action⁷. The data are more consistent with our hypothesis^{8,9,12} that phytochrome is a normal component of the membrane where it acts as a permease, or transport factor, to regulate the movement of one or more critical metabolites between compartments.

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Regulation of RuDP carboxylase/oxygenase activity and its relationship to plant photorespiration

RIBULOSE 1,5-diphosphate carboxylase/oxygenase (fraction 1 protein), the most abundant protein in nature, is a key photosynthetic enzyme catalysing the addition of CO₂ to ribulose 1,5-diphosphate (RuDP) to form two molecules of phosphoglyceric acid (PGA). The enzyme also has a competitive oxygenase function catalysing the splitting of RuDP by O₂ to form one molecule of PGA and one molecule of phosphoglycolate^{1–3}. The latter function of this enzyme has been suggested to be the primary source of phosphoglycolate, the principal substrate for photorespiration⁵.

Fraction 1 protein consists of eight large and eight small subunits⁶. The large subunits are both encoded and synthesised within the chloroplast^{7,8} and seem to function as

the catalytic sites for the carboxylase/oxygenase functions of this enzyme⁹. In contrast, the small subunits are both encoded and synthesised outside the chloroplast and their function is unresolved¹⁰. In this report, we present evidence that the small subunits regulate the carboxylase/oxygenase activity of fraction 1 protein⁸ and that the oxygenase function of this enzyme is probably not the principal source of phosphoglycolate *in vivo*⁵.

We prepared crystallised fraction 1 protein¹¹ from green, wild-type (*su/su*) tobacco leaves (cv John Williams Broadleaf) and from the heterozygous, dominant, yellow mutant (*Su/su*) of this line. Mature leaves were obtained from green and yellow progeny derived by selfing the heterozygous yellow plant. Two important features of the yellow plants are that they result from a nuclear mutation¹² and that yellow plants have a two to threefold higher rate of photorespiration than the wild-type sibling^{13,14}.

The polypeptide patterns of the fraction 1 protein isolated from the wild type and yellow mutant sibling leaves are shown in Fig. 1. Both proteins had identical isoelectric focusing patterns consisting of three large and two small subunit polypeptides. Thus, we found no detectable (by this technique) structural change of the small subunit between the wild and mutant proteins. As Table 1 shows, however, RuDP carboxylase/oxygenase activities are markedly different. Carboxylase/oxygenase activities of the fraction 1 protein from the mutant were about 50% lower than those obtained with the wild-type enzyme. It should be noted that intermediate levels of activity were observed with a mixture (1:1) of the two enzymes.

A lack of any detectable difference in the small subunit structure between the wild type and its mutant was not entirely unexpected. First, no intraspecific variations in fraction 1 protein have been observed on isoelectric focusing gels in 8 M urea⁷. Second, changes in amino acid composition or sequence without a change in overall net charge of the polypeptides would not be detected by this method.



Fig. 1 Polypeptide composition of fraction 1 protein from wild type (W) and yellow mutant (M) of John Williams Broadleaf, a cultivar of *N. tabacum*. The crystalline fraction 1 proteins were s-carboxymethylated before applying to a prefocused 4.5% polyacrylamide slab gel containing 1% ampholine (pH 5–7) and 8 M urea. Isoelectric focusing was performed as described previously¹⁵.

Table 1 RuDP-carboxylase/oxygenase activities of crystallised fraction 1 protein from John Williams Broadleaf wild type (*su/su*) and yellow mutant (*Su/su*) leaves

Experiment	Fraction 1 protein	Carboxylase activity* (c.p.m. $\times 10^2$ per mg protein)	Relative carboxylase activity (%)	Oxygenase activity† (nmol O ₂ per mg protein per min)	Relative oxygenase activity (%)
1	Wild type (<i>su/su</i>)	414.3	100	25.8	100
	Mutant (<i>Su/su</i>)	236.5	57	10.1	39
	Mixture (<i>su/su</i> : <i>Su/su</i>)	317.2	76	17.0	66
	(1:1 mixture)				
2	Wild type (<i>su/su</i>)	425.0	100	28.3	100
	Mutant (<i>Su/su</i>)	242.0	56	11.4	40

Experiments 1 and 2 were done with crystalline fraction 1 protein obtained from separate progeny.

*RuDP carboxylase activity was determined by the $\text{H}^{14}\text{CO}_3^-$ fixation assay¹⁶.

†RuDP oxygenase activity was measured polarographically¹⁷.

Recent results indicate that serological methods can distinguish differences in fraction 1 protein polypeptide composition not resolved by the isoelectric focusing technique (J. C. Gray, personal communication).

The difference in carboxylase and oxygenase activity observed for crystallised fraction 1 protein from the wild type and yellow mutant variety of tobacco was highly reproducible (Table 1). It should be noted that the yellow plant is a nuclear mutant and that it is the small subunit of fraction 1 protein which is nuclear encoded⁷. The difference in activities cannot be attributed to changes in the large subunits (which are maternally encoded) since the proteins were prepared from progeny derived from one parent. Any maternal influence should be expressed equally in all siblings. Thus, our results suggest that the small subunit modifies or regulates enzymatic activity of this protein. How this is accomplished remains to be established.

A surprising outcome of these experiments was the apparent lack of complete correlation between the specific carboxylase/oxygenase activities of the isolated fraction 1 proteins and the respective physiological responses of the plant. For example, the yellow mutant, grown under moderate intensities of light and normal atmospheric levels of CO₂, characteristically has a higher photorespiratory rate (~threefold) and a lower net rate of photosynthesis, compared with the wild type^{13,14}. We note from the data in Table 1 that (1) the carboxylase and oxygenase activities of fraction 1 protein seem to be tightly coupled functions; (2) the carboxylase activities of the isolated enzymes and the respective photosynthetic yields of the parent plants are correlated, and (3) the oxygenase activities of the isolated fraction 1 proteins and the respective photorespiratory rates of the parent plants are not correlated. Based on these data, we suggest that the small subunit regulates the oxygenase and carboxylase function of fraction 1 protein concomitantly rather than independently. Lorimer and Andrews¹⁸ earlier proposed on theoretical grounds that the two functions of this enzyme could not be separated. Our results also support the proposal that RuDP-oxygenase is not the sole source of phosphoglycolate⁵.

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Increase in serum haptoglobin stimulated by prostaglandins

SERUM haptoglobin is decreased in haemolytic anaemias and severe hepatic dysfunction such as cirrhosis, and increased in many pathological states, especially inflammatory and malignant neoplastic disorders¹. Serum haptoglobin can be increased markedly in experimental animals by various inflammatory agents such as turpentine, carrageenan or proteolytic enzymes². Others, such as histamine and 5-hydroxytryptamine, produce a very slight increase³. I have now found that various prostaglandins (PGE₁, PGE₂, PGF_{2α} and PGA₂) can increase serum haptoglobin as much as do turpentine and carrageenan.

Willis⁴ showed that PGEs are increased in inflammatory exudate, and Hammerström *et al.*⁵ found increased PGE₂ in fibroblasts transformed by polyoma virus. In my experiments PGE₁ (as little as 1 µg kg⁻¹) produced more than a fivefold increase of serum haptoglobin in rabbits; 2 µg kg⁻¹ produced more than a tenfold increase 24 h after injection (Fig. 1a). A larger dose produced a more marked response. Repeated injection produced a prolonged increase, as in pathological states (Fig. 1b). This response differs from that observed in response to turpentine, which had the same effect when injected repeatedly as when injected once².

The effect of PG was inhibited by previous administration of cycloheximide, 5-fluorouracil or ethionine (Fig. 1c), which suggests that PG stimulates the synthesis of haptoglobin in the liver. On the other hand, the action of turpentine was not inhibited by these agents (Fig. 1c), suggesting that turpentine releases stored haptoglobin in the liver. Only previous treatment with carbon tetrachloride inhibited the action of turpentine^{6,7}. Carbon tetrachloride seems to block the passage of haptoglobin from the hepatic pool into the circulation⁸. PGE₁ increases the

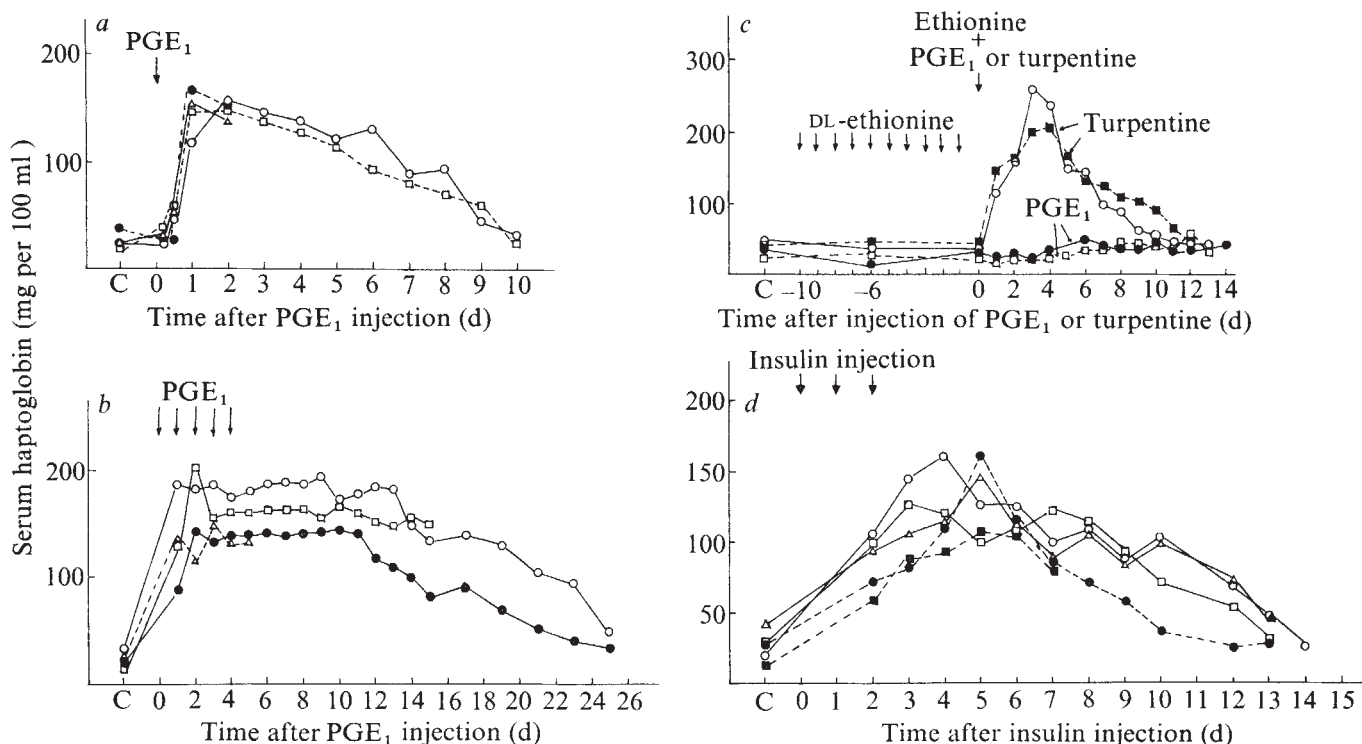


Fig. 1 *a*, Effect of intravenous PGE₁ (2 µg kg⁻¹) on serum haptoglobin level in four rabbits. PGE₁ (5 mg) was dissolved in 0.5 ml of 95% ethanol, and mixed with 4.5 ml of 0.02% Na₂CO₃ solution. The stock PGE₁ solution (1 mg ml⁻¹) was divided into samples of 0.05 ml (50 µg of PGE₁) to vials, and kept frozen until use. The stock PGE₁ solution was diluted with 0.15 M NaCl immediately before use. Serum haptoglobin was measured by paper electrophoresis¹³. Maximum haptoglobin was reached after 24 h and returned to control level after 10 d. The pattern is very similar to that observed after injection of turpentine. PGE₁ (30 µg kg⁻¹) increased rabbit serum haptoglobin as much as 300 mg per 100 ml of serum. When PGE₁ was administered subcutaneously, the effect was much less prominent. A similar effect was obtained with PGE₂, PGF_{2α} or PGA₂ but with less marked effect. *b*, Effect of prolonged administration of PGE₁ (2 µg kg⁻¹ for 5 d) on serum haptoglobin in four rabbits. *c*, Effect of DL-ethionine (10 mg kg⁻¹) on PGE₁ and action of turpentine on serum haptoglobin. Ethionine was administered intraperitoneally to four rabbits for 10 d. On the eleventh day PGE₁ (30 µg kg⁻¹) was injected with ethionine into two rabbits. The remaining two rabbits were injected with 1 ml of turpentine subcutaneously at the dorsolumbar region. Cycloheximide (2 mg kg⁻¹) was injected for 5 d and 5-fluorouracil (10 mg kg⁻¹) was injected intraperitoneally. *d*, Effect of insulin (0.5 U kg⁻¹ d⁻¹), administered intramuscularly for 3 d.

content of cyclic AMP in the liver, whereas PGF_{2α} and PGA₁ have no effect^{9,10}. Insulin, which causes a decrease of cyclic AMP in the liver¹¹, also increased serum haptoglobin quite strikingly in my experiments (Fig. 1*d*). Thus PG may stimulate haptoglobin synthesis directly in the liver, or act in some other way not involving cyclic AMP.

Whereas the biological actions of PGs differ according to the type of PG and tissue involved¹², the fact that serum haptoglobin was increased by every PG tested, irrespective of structure, is interesting. In any case, serum haptoglobin level provides a convenient marker for various actions of PG *in vivo* as long as the function of the liver is intact. The biological significance of increased haptoglobin in the serum remains to be elucidated.

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Exclusively inhibitory action of iontophoretic acetylcholine on single neurones of feline thalamus

IN most regions of the mammalian brain only a small percentage of the neurones located with multi-barrelled micropipettes are inhibited by iontophoretic acetylcholine (ACh). Indeed, the much more frequent occurrence of cells excited by rather small doses of acetylcholine (see ref. 7 for review), together with the finding that both the inhibitory and excitatory actions of ACh are often antagonised by the same pharmacological agents, has led to the suggestion that the inhibitory action of ACh is mediated indirectly through the excitation of neighbouring cholinceptive interneurons whose inhibitory transmitter is a substance other than ACh^{2,3}. Several workers⁴⁻⁸ have tried to overcome this difficulty by working in the most superficial layers of the cerebral cortex where a strong inhibitory action of ACh predominates and ACh excitation has seldom been reported. We wish to draw attention to the possibility that the nucleus reticularis of the cat thalamus may well prove to be an appropriate area in which to study the depressant action of ACh on central mammalian neurones since almost every cell encountered proved to be inhibited by a short, low amplitude pulse of ACh.

Four cats anaesthetised with a mixture of halothane (0.5%), nitrous oxide (65%) and oxygen were placed in a stereotaxic frame and burr holes prepared in both sides of the skull at the appropriate stereotaxic coordinates. Through each burr hole a single track was made using a freshly filled five barrelled micropipette which contained one barrel filled with 4 M NaCl for single unit recording, another with 1 M NaCl for current and current balancing controls and two drug barrels containing 1M AChCl and 1 M sodium glutamate. In this preliminary study no other drugs or putative inhibitory transmitters were present in the microelectrodes. The coordinates of the electrodes were AP9.5 and L7.0 or 8.0 and recording began at H7.0 and continued until H0.0 (ref. 9). After a single microelectrode track had been made on each side of the brain the animals were more deeply anaesthetised with pentobarbitone, killed by perfusion fixation, and the brains prepared for histological analysis.

In Fig. 1*b* five microelectrode tracks, made in four cats on the stereotaxic coordinates AP9.5 and L7.0 or 8.0, are represented schematically by vertical lines to show the depth of the neurones which were either inhibited (●) or excited (×) by small iontophoretic applications of ACh as the microelectrodes transversed first the nucleus reticularis and then entered the ventrobasal complex of the thalamus. Track 1, which lay within the thickness of a single histological section and from which the line drawn in Fig. 1*a* was traced, penetrated only the nucleus reticularis and located only cells inhibited by ACh. All other tracks lay slightly more medial to track 1 and penetrated both the nucleus reticularis and the ventral basal complex of the thalamus. Tracks 4 and 5 are from the same cat. In track 4 the results suggest that the electrode re-entered the nucleus reticularis at the lower limit of its descent and again located cells inhibited by ACh. During the withdrawal of this electrode through the nucleus reticularis three more

cells inhibited by ACh (○) were found. During a subsequent descent of the same electrode on the right side of the cat (track 5), the depth distribution of cells inhibited and excited by ACh was almost identical to that seen in track 4. This experiment eliminates the possibility that the change from ACh-evoked inhibition to excitation reflects a progressive deterioration in either the health of the animal, or the effectiveness of the microelectrode and its contents. The oscillographs in Fig. 2 illustrate the changes in single unit activity of two neurones located in track 4 during iontophoretic applications of ACh. The first cell located in the nucleus reticularis at the approximate stereotaxic coordinate H4.41 shows the typical spontaneous activity of all cells located in this region and was strongly inhibited by ACh. The second neurone which lay approximately 2.43 mm deeper than the nucleus reticularis shows the typical excitation evoked by iontophoretic ACh applied to a majority of neurones in the ventrobasal thalamus¹⁰. The ACh-evoked inhibition could not be mimicked by the passage of larger amounts of positive current through the NaCl barrel of the microelectrode and was only marginally reduced in intensity when the current through the ACh barrel was balanced by passing a negative current of equal magnitude through the NaCl barrel. Of 74 cells found to be inhibited in the five tracks shown schematically in Fig. 1*a* (cells less than 50 μ m apart and showing the same response to ACh are represented by a single symbol) 17 were inhibited when the retaining current on the ACh barrel (25 nA) was reduced and 57 more by currents of less than 40 nA (33 ± 3 nA).

In addition, the inhibition evoked by the release of ACh by relatively low current applications had a shorter latency of onset than the excitations evoked by either the same or larger currents from the same microelectrode during its descent through the deeper nuclei of the thalamus. The observations that the onset of ACh-evoked inhibition is of

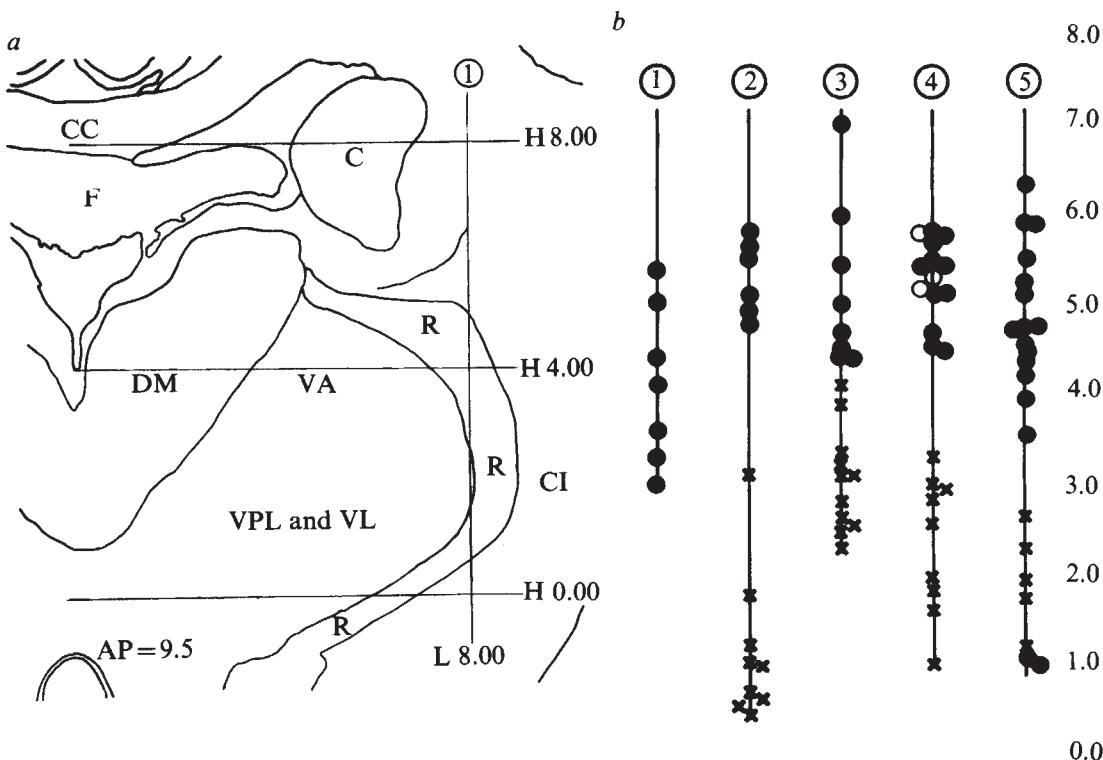


Fig. 1 Schematic drawings to show how cells located within the nucleus reticularis were inhibited by iontophoretic ACh and those lying deeper in the ventrobasal complex of the thalamus excited. *a*, From the histological section which contained the microelectrode track represented by line 1 in *b* shows line L8.00 down which this particular microelectrode passed. The five vertical lines in *b* schematically show the depth distribution of the cells which were either inhibited (●) or excited (×) as microelectrodes penetrated or were withdrawn (○) from the brain of four cats along the coordinates AP9.5 and L7.0 (2 and 3) or 8.0 (1, 4 and 5). Tracks 4 and 5 are from the left and right side of the same cat. The depths of the cells along each track were read from the micrometer of the micromanipulator drive and no attempt has been made to correct these values so that they correspond with the coordinates taken from the atlas of Snider and Niemer⁹ and arbitrarily assigned to the line drawing.

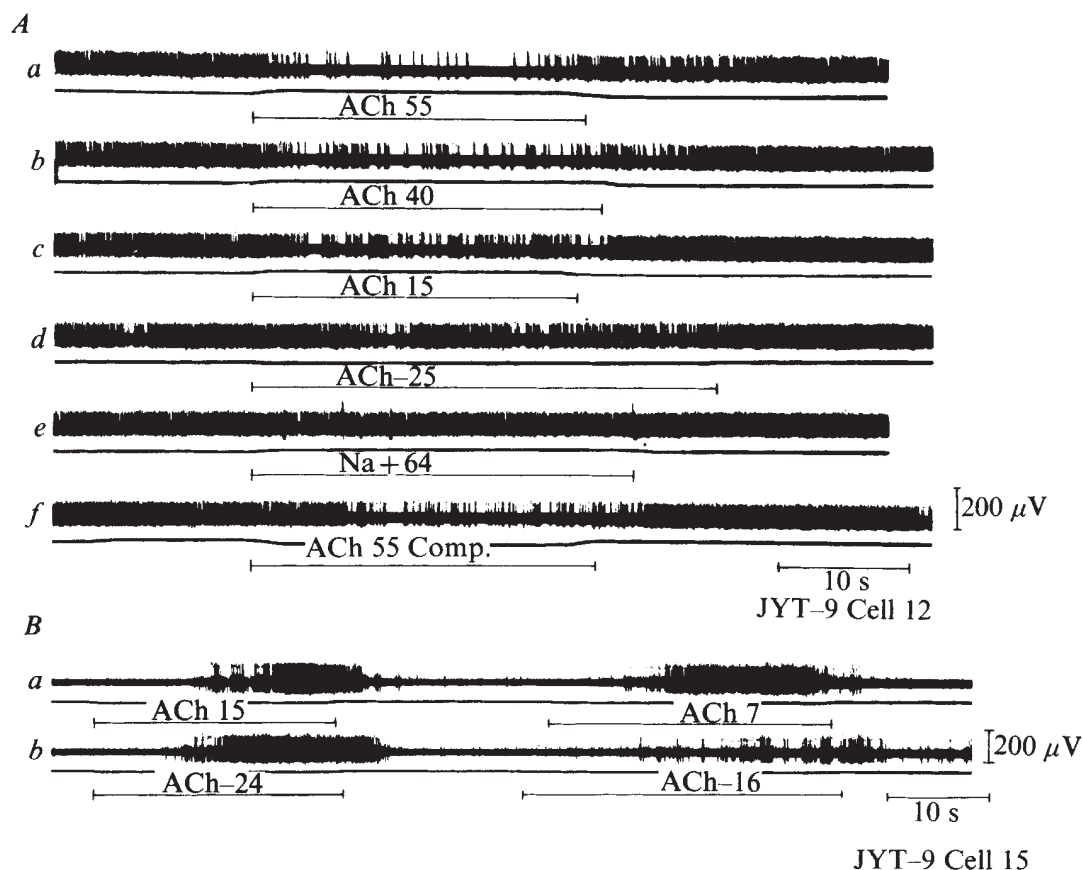


Fig. 2 Oscillographic records to show the inhibition and excitation of neurones in the nucleus reticularis and ventrobasal complex of the thalamus located in the same microelectrode track. *A*, From a cell located at the stereotaxic coordinate H4.41 showing: *a-c*, dose-related inhibitions evoked by progressively smaller doses of ACh released by currents from 55–15 nA; *d*, effect of withdrawing 25 nA of the 25 nA backing current; *e*, lack of effect of 60 nA positive current passed through NaCl barrel; *f*, effect of balancing the 55 nA passed through the ACh barrel by -55 nA passed through the NaCl barrel. *B*, Records *a* and *b* show excitation of a neurone located 2.43 mm deeper in the track by similar doses of ACh. Note that the delay between the onset of the ACh application and the ACh-evoked excitation in *B* was greater than that which occurred in *A* between the onset of the ACh pulse and the ACh-evoked inhibition.

shorter latency than ACh-evoked excitation are in keeping with the view of Krnjević¹, who has suggested that ACh-evoked excitation in the cerebral cortex is often preceded by a period of inhibition. Since in the nucleus reticularis ACh-evoked inhibition has a shorter latency than the excitation evoked by larger currents in the ventrobasal complex, it is difficult to believe that ACh-evoked inhibition could be mediated by the excitation of an intervening cholinergic interneurone. The possibility that the inhibitory action of ACh might be mediated by a presynaptic mechanism was eliminated in part by showing ACh to be an equally effective inhibitor of cells when firing was enhanced by glutamic acid.

The finding that the action of ACh in the thalamus is excitatory in one region and inhibitory in another could be of great functional significance. For instance it now seems possible that a single cholinergic pathway could excite neurones of the ventrobasal complex and inhibit neurones of the nucleus reticularis. This 'push-pull' arrangement is in keeping with the neurophysiological data of Schlag and Wazak¹¹ and Massion and Rispal-Padel¹², which has led to the suggestion that the firing of the neurones of the ventrobasal complex is modulated by inhibitory interneurones whose cell bodies lie within the nucleus reticularis. This idea is contrary, however, to Jasper's¹³ original hypothesis which supposed that the nucleus reticularis was the 'final relay' nucleus of the ascending excitatory pathway involved in 'cortical arousal'. Moreover, modern anatomical studies of the thalamus have not been able to confirm the presence of a pathway from the nucleus reticularis to the cortex and indeed show the

majority of the neurones of this nucleus to project to the ventrobasal complex of the thalamus^{14–16}. Thus Jasper's original view¹³ that the nucleus reticularis is intimately involved in cortical arousal can only be retained by assuming that the neurones of the nucleus reticularis are inhibited by the ascending cholinergic pathway and thus bring about cortical arousal indirectly through disinhibition of the neurones of the more medial nuclei of the thalamus. Interestingly enough, similar physiological findings^{17,18} have led Krnjević¹ to suggest that the ACh-inhibited cells in the upper layers of the cerebral cortex bring about disinhibition of ACh-excited neurones lying much deeper in the cerebral cortex. An analogous situation exists in the nervous system of *Aplysia*, in which the firing of a single cholinergic neurone has been shown to excite one cell and inhibit another^{19,20}.

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Formation of oestradiol-17 β from oestrone sulphate by sheep foetal pituitary *in vitro*

OESTROGENS are synthesised in large amounts by the placenta in several species including man and domestic animals. They occur in the foetal circulation as inactive sulphotoconjugates¹ formed by a detoxifying mechanism that protects the foetus from the detrimental effects of high levels of these potent hormones. In the adult sheep, oestradiol-17 β exerts feedback effects on gonadotrophin secretion by the adenohypophysis, as in other mammals². In the foetal lamb the pituitary and adrenal are essential in the onset of parturition for hypophysectomy results in the prolongation of gestation, and the infusion of adrenocorticotrophic hormone (ACTH) or glucocorticoids induce delivery³. The factors that regulate the activity of the foetal adenohypophysis in late pregnancy are unknown.

A study of the steroid metabolising capacity of the foetal and maternal adenohypophysis and hypothalamus has revealed that oestrone sulphate, present in foetal circulation, can act as a prohormone since it is readily converted *in vitro* into the more biologically active oestrogens, oestrone and oestradiol-17 β , especially in the foetal adenohypophysis.

Ewes were anaesthetised with fluothane and foetuses were removed by Caesarean section. After exsanguination under anaesthesia the maternal and foetal adenohypophysis, hypothalamus and a portion of the frontal cortex were removed and weighed. Tissue was minced and incubated in 2 ml of buffer (0.02 M Tris-HCl, 0.32 M sucrose, 0.001 M

MgCl₂ and 0.005 M NADPH, pH 7.4) at 37 °C for 2 h in 95% oxygen, 5% carbon dioxide. Incubations contained 1 μ Ci of either 6,7-³H-oestrone sulphate (2.13 nmol, 470 mCi mmol⁻¹) or 6,7-³H-oestrone (0.022 nmol, 45 Ci mmol⁻¹). After incubation labelled oestrogens were isolated by phenolic extraction, solvent partition and thin-layer chromatography and their radiochemical purity was established by recrystallisation to constant specific activity.

³H-oestrone sulphate was hydrolysed to oestrone and converted to oestradiol-17 β in all tissues studied (Tables 1 and 2). The arylsulphatase enzymatic activity was about three times greater in the adenohypophysis of the foetus than in that of the ewe ($P < 0.02$). The activity in the hypothalamus and the frontal cortex of both foetus and ewe was low, being similar to that observed in the maternal adenohypophysis. In all tissues, labelled oestrone and oestradiol-17 β formed from oestrone sulphate accounted for the total radioactivity recovered in the phenolic fraction. There was no evidence of sulphotransferase activity in any of the tissues studied when ³H-oestrone was used as substrate.

The quantity of unconjugated oestrogens produced from ³H-oestrone sulphate by the adenohypophysis *in vitro* was substantial. This was exemplified by an experiment at parturition in which foetal pituitary tissue incubated with ³H-oestrone sulphate (423 ng per ml medium) yielded the equivalent of 284 ng oestrone and 12.6 ng oestradiol-17 β from one pituitary during 2 h of incubation. There was no change in the level of arylsulphatase activity of the foetal adenohypophysis during the second half of gestation or at the time of the sharp increase in plasma conjugated oestrone sulphate that occurred shortly before parturition⁴.

The formation of oestradiol-17 β from ³H-oestrone sulphate demonstrated the presence of 17 β -oxidoreductase activity. When foetal and maternal tissues were incubated with ³H-oestrone instead of ³H-oestrone sulphate the activity of the foetal adenohypophysis was found to be approximately seven times that of the maternal adenohypophysis ($P < 0.005$, Fig. 1). In the hypothalamus and frontal cortex of the foetal lamb and ewe, 17 β -oxidoreductase activity was low and similar to that in the maternal pituitary.

The enzymatic capacity of the maternal hypothalamus and adenohypophysis to hydrolyse and convert oestrone sulphate to oestrone and oestradiol-17 β confirms results in anoestrous sheep, and has been implicated in the mechanisms by which pituitary function is regulated⁵. Arylsulphatase and 17 β -oxidoreductase activities in the central nervous system and adenohypophysis of the foetal lamb have not been previously reported. These enzymes may lead

Table 1 ³H-oestrone sulphate metabolised to oestrone and oestradiol-17 β by foetal and maternal tissues from the adenohypophysis and central nervous system

Tissue	Source	Days pregnant	
		81-126	131-145
Oestrone sulphate-oestrone	Foetal	1,121.5 $\pm$ 218.5(3)	1,195.2 $\pm$ 386.6(3)
	Maternal	326.0 $\pm$ 120.4(3)	856.9 $\pm$ 147.6(4)
Hypothalamus	Foetal	703.4 $\pm$ 90.2(3)	794.7 $\pm$ 121.5(3)
	Maternal	653.9 $\pm$ 154.9(3)	856.9 $\pm$ 147.6(4)
Frontal cortex	Foetal	461.8 $\pm$ 121.9(3)	887.8 $\pm$ 62.8(3)
	Maternal	601.7 $\pm$ 51.5(2)	623.5 $\pm$ 178.6(4)
Control, no tissue		< 3.0	< 3.0
Oestrone sulphate-oestradiol-17 β	Foetal	129.1 $\pm$ 22.2(3)	146.9 $\pm$ 50.0(3)
	Maternal	54.8 $\pm$ 22.4(3)	65.4 $\pm$ 45.2(4)
Hypothalamus	Foetal	34.0 $\pm$ 6.4(3)	35.2 $\pm$ 4.2(3)
	Maternal	50.6 $\pm$ 14.5(3)	59.8 $\pm$ 23.3(4)
Frontal cortex	Foetal	20.6 $\pm$ 6.5(3)	26.0 $\pm$ 5.1(3)
	Maternal	27.5 $\pm$ 13.0(3)	25.2 $\pm$ 9.5(4)
Control, no tissue		< 0.6	< 0.6

Figures are pmol per 100 mg tissue per 2 h and represent mean $\pm$ s.e.m.
No. of animals are given in parentheses.

Table 2 ^3H -oestrone and ^3H -oestradiol-17 β formed from ^3H -oestrone sulphate; specific activity during successive crystallisations in acetone-*n*-pentane

			^3H -oestrone (d.p.m. mg^{-1})			^3H -oestradiol-17 β (d.p.m. mg^{-1})				
			1†	2	3	1	2	3	4	5
Adenohypophysis	Foetal*	C	34,379	37,890	37,763	712	440	665	575	725
		ML	45,823	31,132	34,031	576	541	830	548	513
	Maternal	C	59,186	62,465	68,592	4,603	4,887	4,828	4,863	5,293
		ML	78,580	52,854	71,290	4,645	5,012	6,576	4,064	4,204
Hypothalamus	Foetal	C	58,130	68,073	71,844	753	1,081	1,026	1,014	—
		ML	74,564	74,760	66,837	959	1,438	1,000	892	555
	Maternal	C	175,514	163,868	170,409	8,636	8,682	7,916	8,964	8,262
		ML	172,540	192,987	165,821	7,230	7,823	10,880	10,305	12,339
Frontal cortex	Foetal	C	11,236	9,990	13,336	1,217	629	546	469	—
		ML	18,783	10,375	13,098	331	1,112	563	409	533
	Maternal	C	158,664	152,782	170,158	3,883	—	—	—	—
		ML	233,111	163,732	156,596	2,181	—	—	—	—

Oestrogens were isolated from tissue incubations by phenolic extraction, solvent partition and thin-layer chromatography before crystallisation.

*Upper figure crystals (C), lower figure mother liquor (ML).

† Crystallisation number.

—, Not determined.

to the local formation of appreciable amounts of oestrogens from conjugated compounds, such as oestrone sulphate, known to be present in the foetal circulation at a concentration of 2–4 ng ml^{-1} in late pregnancy. Shortly before delivery the plasma concentration of oestrone sulphate in

the foetus increases to even higher levels (up to 39 ng ml^{-1} (ref. 4)), and this change, together with the capacity of the foetal adenohypophysis to produce oestradiol-17 β , may contribute to the cascade of endocrine events associated with the onset of parturition.

Other workers have postulated that oestrogens formed by aromatisation in the central nervous system and adenohypophysis of the adult rabbit and developing human foetus may be concerned in sexual differentiation, indicated by the higher conversion of androstenedione to oestrone in the male than in the female⁶. In the sheep we have found no evidence of aromatisation in similar tissues during the second half of pregnancy, and there was no significant sex difference in the arylsulphatase or 17 β -oxidoreductase activities. It should be noted, however, that sexual differentiation in the sheep occurs during the first third of the 145-d gestation period⁷. The local formation of oestrogens from oestrogen sulphoconjugated compounds seems to comprise an alternative pathway for the production of these potential regulatory hormones within the central nervous system and adenohypophysis.

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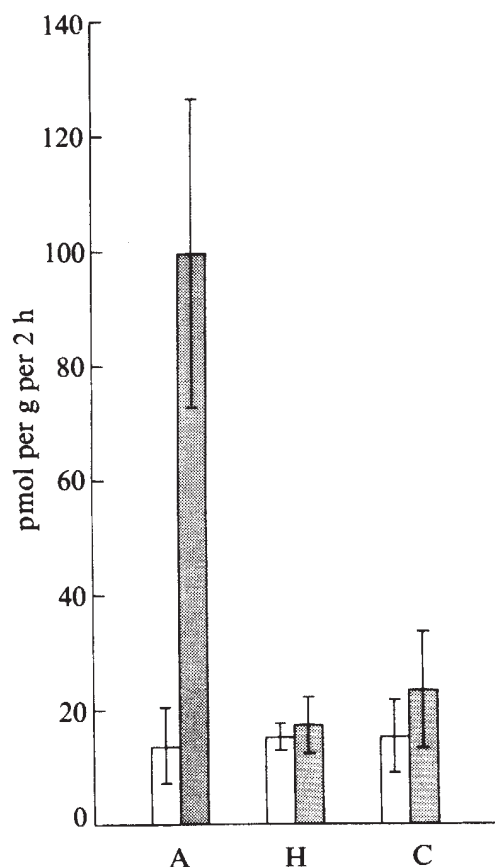


Fig. 1 Formation of oestradiol-17 β from oestrone by the adenohypophysis (A), hypothalamus (H) and frontal cortex (C) of foetal lambs (shaded columns) and adult sheep (open columns) during the last third of pregnancy. The mean $\pm$ s.e.m. of the conversion in four animals is expressed as pmol oestrone converted per 100 mg tissue during a 2 h incubation.

Enzyme induction in mammalian cells defective in 28S ribosomal RNA formation

SYNTHESIS of ribosomal RNA and its transfer to the cytoplasm have been implicated repeatedly in the processing and transport

of informational RNA and the regulation of enzyme activities¹⁻⁴. We have studied the involvement of rRNA in the RNA synthesis-dependent induction of the microsomal enzyme, aryl hydrocarbon hydroxylase⁵; the activity of this mixed-function oxygenase in mammalian tissues and in cells in culture increases many-fold after a few hours exposure to a polycyclic hydrocarbon^{5, 10}. The induction process requires *de novo* protein synthesis and DNA-dependent RNA synthesis^{5, 9, 11} and can be influenced by post-transcriptional regulatory mechanisms^{9, 12}.

Our observations suggested that the induction-specific RNA is a heterogeneous, informational RNA which is transferred to and functions in the cytoplasm independently of concomitant rRNA synthesis and transfer⁵. These observations were based on the use of the inhibitors actinomycin D and cycloheximide, both of which may interfere with several aspects of the regulation of aryl hydrocarbon hydroxylase activity^{5, 9, 11, 12}. To avoid the ambiguities associated with the use of the inhibitors, we have now examined the possible role of newly formed rRNA in the induction process using a mutant cell line of BHK cells which is temperature-sensitive for the production of 28S rRNA¹³.

Mutant BHK-422E cells were obtained from Dr Claudio Basilio and cultured in medium composed of Eagles' minimal essential medium, 5% foetal bovine serum, 10% tryptose broth (Difco), penicillin, streptomycin, tylosin and gentamycin. They were grown at either 34 or 39 °C for 24 h before rRNA synthesis was examined. ³H-uridine was added during a subsequent 2-h incubation and cytoplasmic RNA was extracted and analysed by electrophoresis in polyacrylamide-Agarose mixed gels. In cells grown at the permissive temperature (34 °C), two electrophoretic peaks appeared which corresponded to the 18S and 28S ribosomal RNA (Fig. 1). The amount of newly synthesised 28S RNA was about equal to that of 18S rRNA in cells grown at 34 °C. With longer labelling periods, the radioactivity in the 28S peak increased until it reached twice the amount in the 18S RNA peak (data not shown). At 39 °C, the synthesis and transport of the 18S RNA seemed to be unimpaired, but little newly formed 28S RNA was found in the cytoplasm. Radioactivity in the 28S region was only about 5% of the 18S rRNA in the same cells grown at 39 °C. Similar results were obtained by Toniolo *et al.*¹³ after 20 h observation. The reduced appearance of 28S rRNA

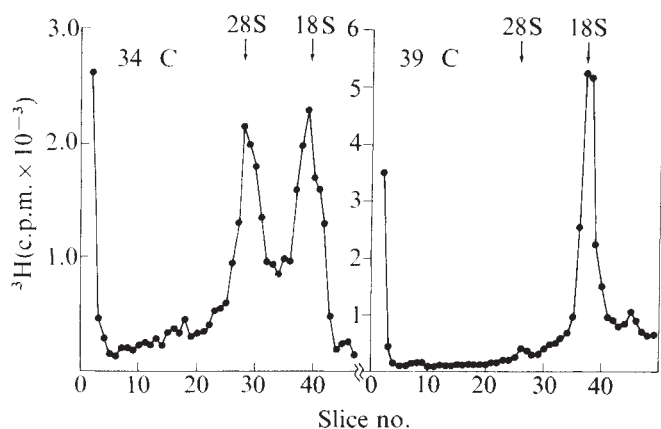


Fig. 1 Electrophoretic analysis of cytoplasmic RNA labelled during growth at 34 and 39 °C. Cultures were kept at 34 or 39 °C for 24 h and incubated for another 2 h in the presence of ³H-uridine (1.0 µCi per ml medium, New England Nuclear). Cells were treated with 0.5% Triton X-100 in phosphate buffer (pH 7.4) containing 1.5 mM MgCl₂. After 20 min, nuclei were centrifuged to a pellet, and the cytoplasmic and nuclear fractions were extracted three times with phenol. A sample of the aqueous phase was added directly to the gel slot and electrophoresed for 90 min in mixed polyacrylamide-Agarose gels¹⁹. Values represent c.p.m. per slice.

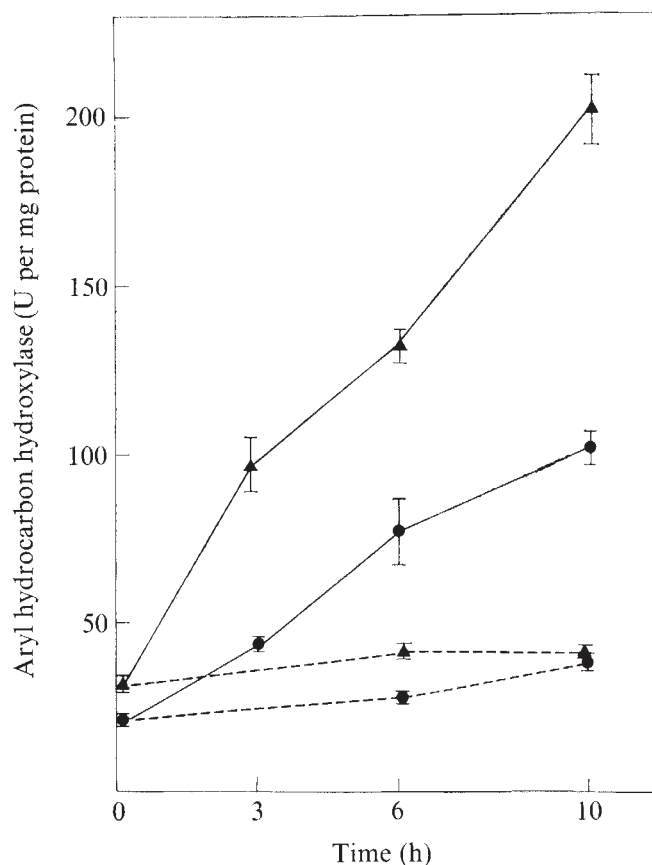


Fig. 2 Induction of aryl hydrocarbon hydroxylase in BHK-422E cells at 34 and 39 °C. Benz(a)anthracene (3 µg per ml medium) was added to cultures after 24 h of incubation at 34 and 39 °C. Cultures were further incubated at the two temperatures and at various times cells from two plates each were pooled. Aryl hydrocarbon hydroxylase activity was assayed by the procedure of Nebert and Gelboin⁷ with minor modifications⁸. The incubation period was 60 min. A 2.0 ml sample of the organic layer was extracted into 1 ml of 1 N NaOH. One unit of aryl hydrocarbon hydroxylase catalyses in 1 min the formation of phenolic product with the fluorescence equivalent of 1 fmol of 3-hydroxybenzo(a)pyrene. Protein concentrations were determined by the method of Lowry *et al.*¹⁸ with ribonuclease A as standard. Values represent the mean and range of duplicate determinations in the absence (---) and in the presence (—) of benz(a)anthracene. ▲, 39 °C; ●, 34 °C.

in the cytoplasm has been attributed to a defect in the processing of precursor 32S rRNA¹³. Electrophoretic analysis of nuclear fractions showed considerable radioactivity in the region of 32S RNA in extracts from cultures incubated at both 39 and 34 °C, but as in the case of cytoplasm, virtually no radioactivity appeared in the 28S region of cultures incubated at 39 °C (data not shown).

The time course of aryl hydrocarbon hydroxylase induction was then examined in the mutant BHK cells at temperatures permissive and non-permissive for 28S rRNA synthesis (Fig. 2). Cultures were set at the respective temperatures 24 h before addition of the inducer, benz(a)anthracene. Hydroxylase activity increased nearly linearly for 10 h at both temperatures. Constitutive hydroxylase activity and inducibility were higher at 39 than at 34 °C. The higher levels of hydroxylase activities at the non-permissive temperature might be an expression of a generally greater metabolic activity at the higher temperature.

The results suggest that aryl hydrocarbon hydroxylase induction occurs in the absence of *de novo* formation of 28S RNA. The ability of the mutant BHK cells to form 28S RNA is lost exponentially with a half time of less than 5 h in cells shifted from 34 to 39 °C (ref. 13). In view of the 24 h preincubation period at 39 °C and the relatively short half life of rRNA

Table 1 Aryl hydrocarbon hydroxylase induction in BHK-422E cells during treatment with actinomycin D and cycloheximide

Group	Treatment*	Incubation period (h)†	Aryl hydrocarbon hydroxylase (U per mg protein)‡
1	Control medium	8	3.9 ± 0.1
2	BA	8	40.3 ± 1.0
3	BA + act D, 2 µg	8	6.5 ± 0.3
4	BA + act D, 4 µg	8	8.3 ± 0.3
5	BA + cycloheximide	8	2.3 ± 0.1
6	BA + cycloheximide followed by BA + act D	3 5	145.3 ± 5.0
7	BA + cycloheximide + act D followed by BA + act D	3 5	19.1 ± 0.5

*Cultures were treated with benz(a)anthracene (BA), 3 µg in 3 µl of dimethylsulphoxide (DMSO) per ml medium (BA, groups 2–7) or with 3 µl of DMSO (control medium, group 1). Cycloheximide was used at 10 µg ml⁻¹ (groups 5–7), actinomycin D (act D) at 1 µg ml⁻¹ (groups 6–8).

†Cultures of groups 6 and 7 were treated for an initial period of 3 h. The medium was then changed and cultures were treated for a further 5 h as indicated.

‡Mean and range of duplicate determinations.

in the nucleus^{14,15}, it seems unlikely that significant amounts of unlabelled 28S rRNA are transferred to the cytoplasm. It is also unlikely that the small residual 28S rRNA production at 39 °C (Fig. 1 and ref. 13) has significance in the enzyme induction. This would imply that either 28S rRNA is produced in excess over that needed for translation of essential proteins at the permissive temperature, or that a specific induction-related 28S rRNA is preserved at the non-permissive temperature.

The requirement for RNA synthesis during aryl hydrocarbon hydroxylase induction in the mutant BHK cells at 39 °C is shown in Table 1: actinomycin D (2 or 4 µg ml⁻¹ medium) inhibited the increase in enzyme activity during 8 h (compare groups 1–4); after a block of protein synthesis in the presence of the inducer which then is followed by release of protein synthesis and a block of RNA synthesis, the enzyme activity increased more than 30-fold (group 6). This enhanced induction is largely suppressed when actinomycin D is present during the initial block of protein synthesis (group 7). The phenomenon of enhanced induction after a temporary block of protein synthesis is discussed elsewhere^{9,16}. The results indicate that aryl hydrocarbon hydroxylase induction in BHK-422E cells at 39 °C, as in other cells in culture at 37 °C (refs 5, 9, 11 and 12), depends on a transient *de novo* RNA synthesis which may be independent of concomitant protein synthesis.

Enzyme induction in the virtual absence of 28S rRNA formation is interesting in two aspects. First, it has been suggested that the rate of rRNA synthesis, maturation and transfer have a role in adaptive changes of enzyme activities during cellular growth, differentiation and hormone action^{1,2}. This is not supported by the results presented here, although rRNA requirements may differ between the highly selective, substrate-mediated induction of the hydroxylase and the broader regulatory processes affected by hormones. Second, rRNA synthesis and transfer have been implicated in the control of mRNA synthesis and expression^{3,4}. Induction of aryl hydrocarbon hydroxylase clearly requires the synthesis of a specific RNA^{5,9,11}. The effects of preferential inhibition of rRNA synthesis⁵ or of heterogeneous RNA synthesis¹⁷ during induction suggested the involvement of an RNA of the heterogeneous informational type. In agreement with earlier observations⁵, the results indicate that this induction-specific RNA is synthesised and transferred to the cytoplasm independently of concomitant 28S RNA formation and transfer. The results do not exclude the possibility that a nucleolar function is essential in the flow of information from the nucleus to the cytoplasm as postulated by Harris *et al.*^{3,4}, but the processing and transfer of 28S RNA now can be eliminated from consideration in the search for this function.

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Compact form of DNA induced by spermidine

RECENT studies have revealed that the packaging of DNA into phage heads is an ordered sequence of structural and biochemical events rather than a simple, spontaneous self-assembly of component molecules. In the assembly of T7 for example¹, heads containing the products of genes 8–10 and 14–16 are apparently formed first. Gene 9 protein is removed from the heads before or during the incorporation of DNA. At least two other gene products which play no direct structural role are required for the maturation of the heads. A somewhat similar situation exists for phage λ, which has been shown to require ATP for the *in vitro* packaging of DNA into preformed heads (petite λ)².

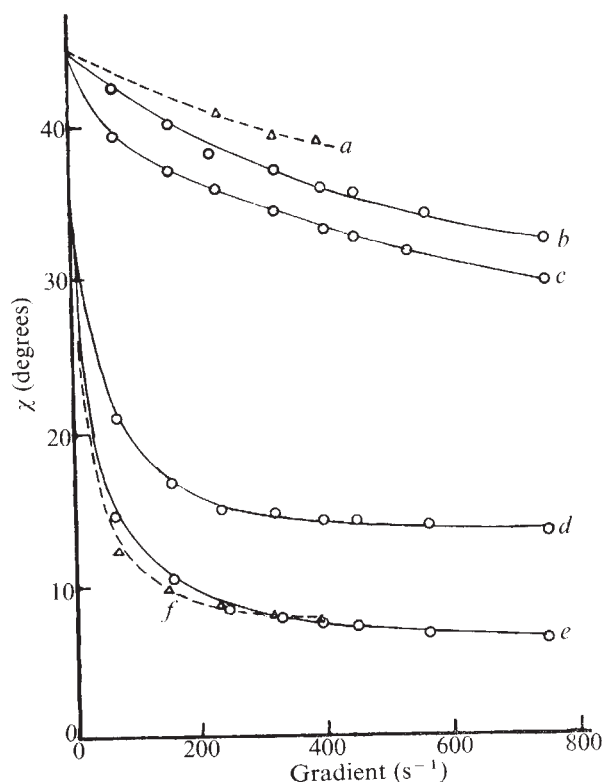
Apart from the mechanistic description of the specific processes of DNA packaging, there is the important question of whether these processes are facilitated by a thermodynamic driving force toward the compaction of DNA. Packaging *in vitro* DNA (that is, DNA in 0.2 M ionic strength with a small amount of buffer) without some generalised driving force would be so disadvantageous thermodynamically that it does not seem to be a feasible process. For example, T7 DNA may be represented by an effective ellipsoid³ with a volume of ~10¹² Å³ (ref. 3). This is roughly 10,000 times the volume of the T7 phage head. A compression of this magnitude would result in a considerable opposing pressure. In addition to this essentially entropic force, there would also be the work required to bring the negatively charged segments of DNA into close proximity to one another and the energy required to bend or fold the stiff DNA chains into a compact structure. If packaging were to take place with all of these opposing forces in full operation, not only would a great deal of free energy be required for the process, but the phage would be 'spring-loaded' to an increasing degree during the packaging and would expel the DNA spontaneously if there were any relaxation in the packaging mechanism. If by contrast phage particles are formed in an ambience in which these forces are overcome or strongly diminished, the agents responsible for the packaging would have the simpler role of selecting a proper mechanistic pathway for a process which is essentially spontaneous.

The notion that the free energy of the compacted form is lowered by its interaction with polyamines⁴, Mg^{2+} , (ref. 5) cationic polypeptides⁶, or other reagents has been in the literature for some time. Lerman *et al.*⁷ have shown that another possible way of favouring the collapse of DNA is by raising the free energy of the expanded form. This is accomplished by the addition of high polymers which interact unfavourably with DNA. The condensed form is simultaneously stabilised by the presence of high salt concentrations. DNA condensed in this manner (ψ DNA) has also been studied by Evdokimov *et al.*⁸ and by Laemmli *et al.*⁹ and it seems to be well established that this treatment in sufficiently dilute solution converts single molecules of DNA into compact, dense particles.

It was our desire to see if the same effect could be achieved using materials which are known to exist in host bacterial cells, in particular, the polyamines. This idea is relatively old—for example an early investigation by Kaiser *et al.*⁴ indicated that DNA in the presence of spermine is converted to a form which is protected from shear. The reason that the notion has not been pursued further presumably lies in the difficulty of studying such systems¹⁰. In terms of polymer theory¹¹, conditions are required in which segmental attraction is strong (Flory-Fox parameter $\alpha < 1$) and these are precisely the conditions which are well known to cause aggregation and precipitation. The preventative against this is very low concentration. Lerman's group¹² was able to demonstrate monomolecular collapse by the sedimentation of radioactive DNA which was converted into the ψ form in extremely dilute solution. In general, a physical method of observing the collapse is difficult to find since most techniques become inoperative at the dilutions required.

The technique selected for this investigation is streaming linear dichroism. A brief description of the apparatus has been published¹³ and the details will be published soon¹⁴. The

Fig. 1 Variation of isocline angle with gradient for T7 DNA in presence of various concentrations of spermidine. Spermidine concentrations (mol l^{-1}), letters beside curves. Buffers in all cases consisted of 1 mM NaCl + 1 mM Na cacodylate, pH 6.6, temperature, 20.0 °C. DNA $A_{260} = 0.1$ except for points designated Δ , for which $A = 0.006$. M ($\times 10^{-3}$): a, 5.0; b, 7.5; c, 5.0; d, 4.4; e, 2.5; f, 0.0.



procedure is the same as for streaming birefringence except that linear dichroism (LD) at 254 nm is used for finding the angle of isocline, χ . The strong absorptive properties at 254 nm combined with stress modulation and lock-in amplification provides great sensitivity for the measurement of both χ and the magnitude of the LD. Moreover, stream orientation is extremely sensitive to molecular dimensions. With this technique it is possible to make measurements on DNA where aggregation can be largely forestalled.

The effect of spermidine concentration on the orientational properties of DNA is shown in Fig. 1 which plots the isocline angle against gradient. In the conditions of the experiment (0.001 M NaCl, 0.001 M Na cacodylate, pH 6.6), there is an abrupt change in properties at about 4.6×10^{-5} M spermidine.

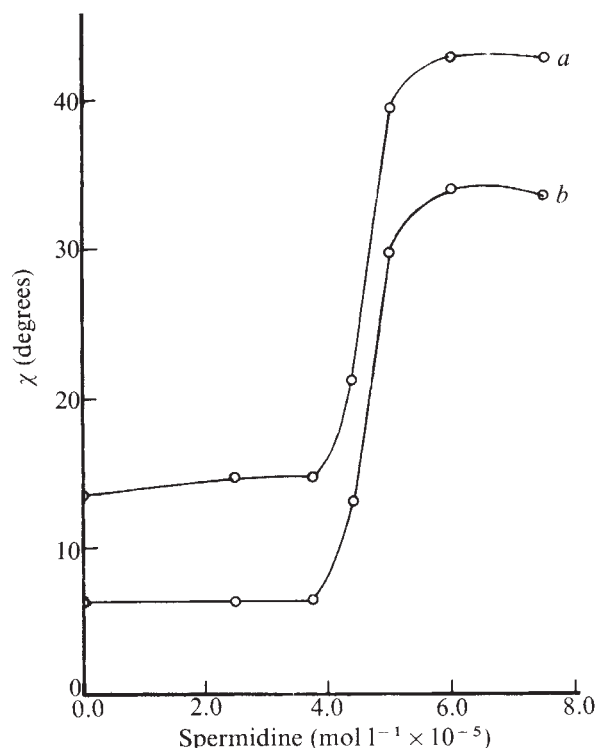


Fig. 2 Variation of isocline angle of T7 DNA with spermidine concentration at extreme values of gradient: a, 74.2 s^{-1} ; b, 742 s^{-1} . Conditions as in Fig. 1. DNA $A_{260} = 0.1$.

These results are indicative of a drastic cooperative change in size and shape. The effect is brought out more clearly in Fig. 2 which plots the angle of isocline against spermidine concentration for two fixed velocity gradients. The lower curves of Fig. 1 are typical for a long flexible molecule which is being simultaneously stretched and oriented by the hydrodynamic field. The upper curves are typical for a molecule of much smaller dimensions with a low degree of orientation in the hydrodynamic gradient. If the molecules were compacted into spheres, there would be no orientation and the LD would vanish. Most of the experiments in Figs 1 and 2 were carried out at a moderately low DNA concentration ($A = 0.1$ in a 1-cm cell). In these conditions, accurate measurements may be made throughout the transition but control experiments with the ultracentrifuge indicate heterogeneity, presumably the result of aggregation. To demonstrate that aggregation is not a necessary part of the phenomenon, experiments were carried out in highly dilute solutions ($A = 0.006$). These solutions showed evidence of aggregation only after a number of hours. A typical experiment on the effect of spermidine on such dilute solutions is shown in Fig. 1 (Δ). Data on the collapsed form are less accurate because of the combination of low concentration and low LD, although the disappearance of native extended DNA is clear cut.

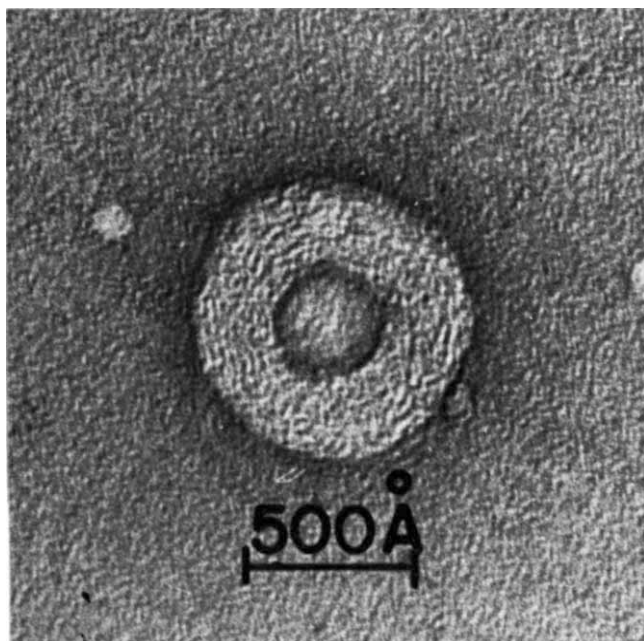


Fig. 3 Electron micrograph of spermidine-collapsed T7 DNA. Carbon-coated copper grids were coated with a monolayer of cytochrome *c* and glow-discharged. Grids were then floated for 10 min on a DNA-spermidine solution, touched to filter paper, floated on 1% uranyl acetate solution and blotted dry. Micrographs were obtained with a Phillips EM300 microscope.

Once the conditions for molecular collapse were established in the LD experiments, a number of ancillary experiments were performed to throw light on the structure of the collapsed form. Figure 3 shows an electron micrograph of a representative particle of DNA ($A = 0.006$) in the presence of 10^{-4} M spermidine in the same conditions of salt and pH as noted for Fig. 1. We consider the general size and shape of these particles to be more significant than the specific morphology since the latter is sensitive to sample preparation. For example, with uranyl acetate staining approximately 90% of the DNA molecules have the morphology of Fig. 3, whereas with unstained, shadowed preparations the molecules have approximately the same size and shape but only 10% have a central cavity. Note that Richards *et al.*¹⁵ have reported the presence of hollow disks in electron micrographs of ruptured T7 phage.

Circular dichroism studies on the collapsed form yielded curves which differ only in negligible details from the B-form curves which are obtained before collapse. This is in contrast to the CD of the ψ form of DNA which is drastically different from the B form, and to the CD spectra of whole phages T5 and T7 which are distorted from the B form. This distortion has been attributed to the presence of C form DNA¹⁶.

Studies of the helix-coil transition of the collapsed DNA-spermidine complex show that the transition is shifted to a higher temperature and is considerably sharper than that of unperturbed DNA.

Experiments with other polyamines revealed that DNA is also collapsed by spermine at significantly lower concentrations than those required for spermidine. A description of these studies plus a more complete interpretation of the LD and electron micrograph experiments will be reported later.

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Evidence for a major conformational change of coat protein in assembly of ϕ 1 bacteriophage

RECENT structural studies^{1,2} indicate that the DNA of filamentous bacteriophages is enclosed in a sheath of α -helical coat protein. In the course of phage biosynthesis this protein is tightly bound to the membrane of the host (*Escherichia coli*)^{3,4}. We present here the results of circular dichroism (CD) studies strongly suggesting that the protein has a quite different conformation in its membrane-bound state, so that a major conformational change must accompany incorporation into the virus.

Methods for the preparation of ϕ 1 and for the purification of coat protein have been described previously⁵. Accurate concentration measurements are necessary for the quantitation of CD spectra. These were made spectrophotometrically, using the following A values, based on dry weight determinations: for whole virus, $A_{260} = 3.78$ or $A_{268} = 3.95$; for purified coat protein, $A_{280} = 1.65$. The values given apply to a solution containing 1 mg ml⁻¹ and a light path of 1 cm. In the experiments using phosphatidylcholine vesicles, where light scattering interfered with spectrophotometric measurements, coat protein containing radioactive tyrosine and lysine was used, specific activity was determined relative to absorbance in the intact phage, and concentration in the vesicles was obtained on the basis of radioactive counts. CD spectra were measured using a Cary 60 spectropolarimeter with CD attachment.

Figure 1 shows the CD spectrum in the peptide absorption region for intact virus and for virus dissociated by 30 mM deoxycholate⁶, but without separation of the components. The possible contribution from DNA to these spectra must be small, both because the DNA content of the virus is small and because the magnitude of the CD spectrum of DNA is low in this spectral range, regardless of conformation⁶⁻⁸. The difference between the curves of Fig. 1 must therefore arise predominantly from a conformational change of the viral protein, more than 98% of which is the viral coat protein.

Mean residue ellipticities of the viral protein obtained from Fig. 1 (assuming that DNA makes no contribution at all) are shown in Fig. 2. A protein content of 88.5% (determined in this laboratory, but agreeing with previous measurements⁹) was used, together with a mean residue molecular weight of 105, calculated from the amino acid composition of the protein. Fig. 2 also shows the CD spectra of chromatographically purified coat protein in 30 mM deoxycholate, of coat protein precipitated by removal of deoxycholate by ultrafiltration and redissolved in 30 mM deoxycholate, and of coat protein similarly precipitated and redissolved in 5 mM oleyl lysophosphatidyl-

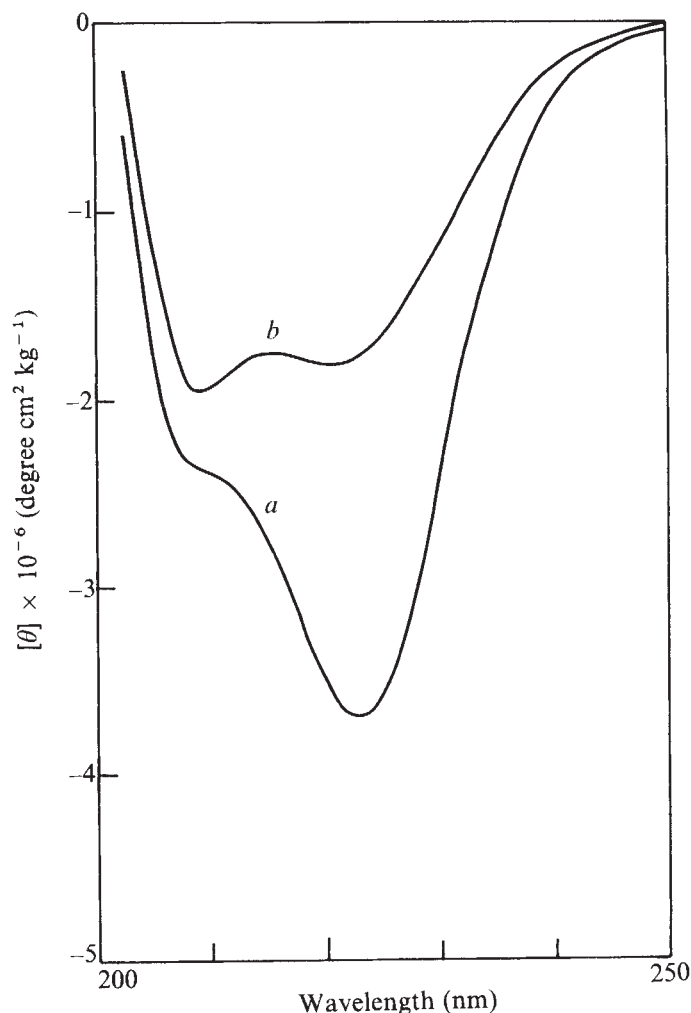


Fig. 1 Circular dichroism (per kg virus) of intact virus in 0.1 M NaCl (a) and after dissociation by 30 mM deoxycholate (b).

choline. (Spectra similar to curves *d* and *e* were obtained in two other detergents as well and in two other micelle-forming lysolipids.) Figure 2 also shows the CD spectrum of coat protein incorporated into egg yolk phosphatidylcholine vesicles. Phage in CHCl_3 -saturated aqueous solution (where the CD spectrum is identical to that of intact phage) was deposited on the walls of a conical centrifuge tube by evaporation of solvent with dry N_2 . Egg yolk phosphatidylcholine dissolved in CHCl_3 was deposited in the same way. Aqueous buffer was added and vesicles were formed by sonication as described by Huang¹⁰ and chromatographed on Sepharose 4B. The coat protein (radioactively labelled) was found to have been separated from viral DNA and eluted with the phospholipid vesicles. The molar ratio of lipid phosphorus to protein in the combined peak was 80. The protein was accessible to proteolytic enzymes, proving that it was not simply trapped in the vesicles during sonication.

The CD spectrum for the coat protein in the intact virus is unlike any known spectrum for a protein or polypeptide in simple aqueous solutions^{11,12}: the peak wavelengths are appropriate for an α -helical chain, but the unequal intensities of the bands at 208 and 222 nm are not. Similar unequal intensities, however, have been frequently observed for proteins in particulate suspension^{13,14} and they have been theoretically explained on the basis of bunching of the absorbing chromophores into a small space, leading to local concentrations far in excess of the average concentra-

tion of the protein or polypeptide in the solution as a whole¹⁵. It is entirely appropriate to observe this effect in the virus, in which over 2,000 protein molecules are contained in a single particle. Since the effect leads to a diminution of intensity, always more pronounced at 208 than at 222 nm, the observed spectrum clearly falls within the range expected for proteins or polypeptides with an α -helical content of 100% (refs 11–13). Our result is thus consistent with the X-ray data cited earlier. It also agrees with previous estimates based on optical parameters^{15,16}.

The CD spectra of amphiphile-solubilised protein are all similar and differ greatly from the spectrum in the intact virus. The helical content is less than 50%. No increase in negative CD is observed below 210 nm, indicating¹¹ that no significant portion of the protein has been converted to a structureless random coil. Part of the non-helical portion of the structure could be in the β -conformation or some twisted variant thereof. If so, it is likely that the hydrophobic amino acid residues (residues 21–39)¹⁷ are involved and this might explain why the coat protein is always in a dimeric state in those detergents in which molecular weight measurements have made⁵, since β structures require interchain hydrogen bonds for stabilisation. Estimates of the location of probable helical segments were made for us

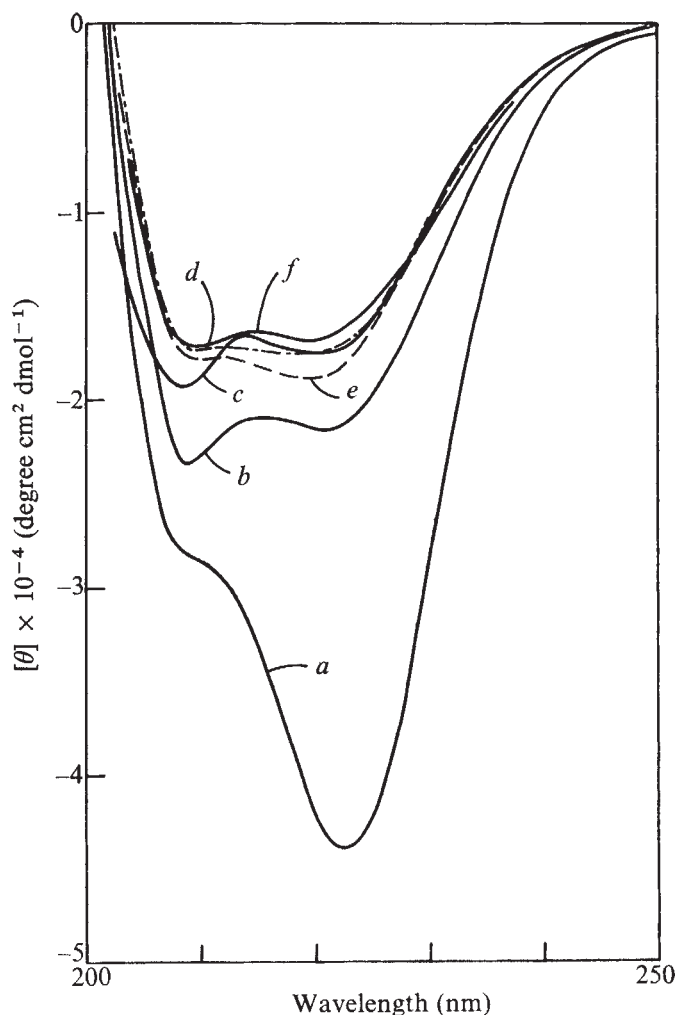


Fig. 2 Mean residue ellipticity of coat protein. *a*, In intact virus, from Fig. 1 assuming no contribution from DNA; *b*, in deoxycholate micelles (30 mM), from Fig. 1 with same assumption; *c*, purified protein in 30 mM deoxycholate; *d*, purified protein redissolved in 30 mM deoxycholate after precipitation; *e*, purified protein in oleyl lysophosphatidylcholine micelles (5 mM) after precipitation; *f*, purified protein in egg yolk phosphatidylcholine vesicles, prepared as described in the text.

by Drs Chou and Fasman and by Drs Krigbaum and Knutton, using the methods they have described previously^{18,19}. Both groups predict helical segments near both chain termini and assign a probable β -pleated structure to residues 26–37 and 27–36, respectively. (The fd coat protein sequence¹⁷ was used for this analysis, but we have found fd and fl to be identical in amino acid composition and the pattern of proteolytic fragments²⁰.)

If we make the reasonable assumption that the similar spectra observed in detergent micelles, phospholipid micelles and phospholipid vesicles also represent the state of the coat protein when bound to the membrane in the host bacterium, and take into account the proteolytic digestion data presented previously²⁰, we are led to the conclusion that the membrane-bound state has the central portion of the coat protein polypeptide inserted into the hydrophobic core of the membrane, very likely in a β conformation. A major structural change must thus occur when the protein is removed from the membrane and incorporated into newly-assembled phage particles.

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Sulphate permease in *A. nidulans* is completely repressed if the mycelium is grown in the presence of cysteine, homocysteine or methionine. Toxicity of chromate, a substrate to the sulphate permease, is therefore easily reversed by the addition of methionine to the growth medium. More than 100 mutants resistant to chromate have been isolated in *A. nidulans*⁸, all of which were allelic, both in heterokaryons and diploids, to the previously known mutant *sB3*, indicating that the mutations affect sulphate permease⁸. One of these mutants, *sB₉₀*, was particularly interesting as, unlike other mutants, it showed a normal growth rate on minimal medium. Chromate (1 mM) inhibited growth of this mutant but 0.03 mM L-methionine was sufficient to reverse chromate toxicity, whereas 5 mM L-methionine was necessary in the case of the wild type. This suggested that the mutant takes up less chromate than the wild type because of altered specificity of sulphate permease or its hypersensitivity to repression. Table 1 supports the second alternative. When the mutant and the wild type were grown in sulphur-free medium (there was enough sulphur in the conidia used for inoculation to ensure growth for 12–16 h), the rate of sulphate uptake in both strains was very similar (see also Table 2). K_m for sulphate was 5.0×10^{-5} M and 4.0×10^{-5} M, respectively, for wild-type and *sB₉₀* strains. A similar inhibition of sulphate uptake by chromate in both strains was observed: $K_i = 3.3 \times 10^{-5}$ M for wild type and $K_i = 3.4 \times 10^{-5}$ M for the mutant *sB₉₀*. These data indicate that the mutation *sB₉₀* does not alter the sulphate permease itself, but rather the regulation of its synthesis. On the other hand, in more than 700 progeny examined from the cross *sB₉₀ × sB3* no wild-type segregants (which would be chromate-sensitive) were found. The *sB₉₀* site must therefore be situated within or very close to the *sB* locus. The simplest hypothesis would be that *sB₉₀* affects a regulatory site involved in the repression of sulphate permease, so that its affinity to a regulatory molecule is increased.

Table 1 Effect of L-methionine in growth medium on level of sulphate permease in *sB₉₀* and wild-type strains

Strains	Sulphate uptake (nmol per min per mg dry weight)		MM—S
	Control MM—S	MM—S + 0.3 mM L-methionine*	MM—S + meth
<i>sB₉₀</i>	5.95	0.47	12.7
Wild type	7.62	2.38	3.2

Mycelia were grown and sulphate uptake assayed as described previously¹⁰. MM—S, minimal medium without sulphur source.

* Minimal concentration that enables strain *sB₉₀* to grow on 1 mM chromate (Table 2).

To test this hypothesis we used a recessive regulatory mutant *suAmeth* in which several enzymes of methionine synthesis including sulphate permease are constitutive⁹. The character of this mutation strongly suggests that this gene is responsible for the synthesis of a repressor-like molecule involved in a negative control system. Due to the constitutive formation of sulphate permease the strain *suAmeth* is extremely sensitive to chromate even in the presence of a high concentration of methionine (Table 2). If the *sB₉₀* mutation causes an increased affinity to the regulatory molecule one would expect to find the mutation *suAmeth* epistatic to *sB₉₀*, as without active repressor the latter mutation cannot be manifested. This was the case. As shown in Table 2 the double mutant *sB₉₀suAmeth* is equally sensitive to chromate as is *suAmeth* alone. As expected, strain *sB3suAmeth* displays the phenotype of *sB3* because even constitutive synthesis of impaired sulphate permease does not lead to an increased chromate uptake. Experiments on sulphate uptake (Table 3) confirm the results of growth tests. Strain *sB₉₀suAmeth* shows a constitutive synthesis of sulphate permease. Thus, the introduction of the *suAmeth* mutation to strain *sB₉₀* results in the changeover from hyper-repressibility to constitutive synthesis

Hyper-repressible operator-type mutant in sulphate permease gene of *Aspergillus nidulans*

SINCE the pioneering work on regulation of the *lac* operon in *Escherichia coli*¹ a great number of operator mutations have been described. In eukaryotic organisms, however, only a few mutations affecting *cis*-acting regulatory element (some of which can be formally interpreted as operator mutants) have been reported^{2–6}. The term 'operator' in the case of eukaryotes is used in an operational sense as it is not known whether it describes a molecular organisation similar to that found in bacteria. This report describes a mutant of *Aspergillus nidulans* altered at a site closely linked to the *sB* gene for sulphate permease⁷. This mutation causes a hypersensitivity of this gene to repression by sulphur amino acids.

Table 2 Effect of *suAmeth* mutation on growth of *sB3* and *sB₉₀* strains on L-methionine (10 mM) and various concentration of chromate

Chromate (mM)	Wide type	<i>sB3</i>	<i>sB₉₀</i>	Strains		
				<i>suAmeth</i>	<i>sB3suAmeth</i>	<i>sB₉₀suAmeth</i>
0.03	+	+	+	+	+	+
0.05	+	+	+	±	+	±
0.06	+	+	+	—	+	—
1.00	+	+	+	—	+	—
1.50	±	+	+	—	+	—
2.00	—	+	+	—	+	—

Growth tests on solid medium (24 h at 37 °C) were carried out as described previously⁸. Growth symbols: +, normal growth; ±, weak growth; —, no growth.

Table 3 Sulphate uptake by *sB3*, *sB₉₀*, *suAmeth* and double mutant strains grown with and without L-methionine

Strain	Sulphate uptake (nmol per min per mg dry weight)	
	MM—S	MM—S + L-methionine (10 mM)
Wild type	10.65	0.00
<i>sB3</i>	0.00	0.00
<i>sB₉₀</i>	10.18	0.00
<i>suAmeth</i>	11.57	8.33
<i>sB3suAmeth</i>	0.00	0.00
<i>sB₉₀suAmeth</i>	12.00	10.13

Culture conditions and sulphate uptake assays were as described previously¹⁰. Djenkolic acid was added to the *sB3* and *sB3suAmeth* cultures as a sulphur source. It does not cause repression of sulphate permease in the wild type.

Table 4 Activity of ATP sulphurylase and homocysteine synthase in *sB3*, *sB₉₀*, *suAmeth* and double mutant strains

Strain	Activity (nmol per min per mg protein)	
	ATP sulphurylase	Homocysteine synthase
Wild type	71.8	35.6
<i>sB3</i>	69.3	32.8
<i>sB₉₀</i>	72.4	48.5
<i>suAmeth</i>	116.8	120.0
<i>sB3suAmeth</i>	152.0	138.5
<i>sB₉₀suAmeth</i>	130.0	154.5

Mycelia for enzyme assays grown, extracted and assayed as described previously¹⁰.

of the sulphate permease; these data confirm both the operator-type nature of the *sB₉₀* mutation and the regulatory role of the *suAmeth* gene product.

One of the most characteristic features of an operator mutation is that it is *cis*-acting. The interpretation of a *cis-trans* test is straightforward in the case of an operator-constitutive mutant which is dominant, but not in the case of a recessive mutant like *sB₉₀*. Diploid *sB₉₀/sB⁺* was sensitive to chromate indicating the recessive character of the mutation.

We have tested two other enzymes that are controlled by the *suAmeth* gene⁹—ATP sulphurylase and homocysteine synthase (Table 4). No significant differences in the level of these enzymes were observed between *sB3*, *sB₉₀* and wild-type strains. Both enzymes were derepressed in the *sB3suAmeth* and *sB₉₀suAmeth* strains. This is additional evidence that the mutation *sB₉₀* affects only the regulation of sulphate permease.

All operator or operator-type mutants described so far in fungi have been selected as having increased activity over that of the wild type, at least in some growth conditions. Recognition of *cis*-acting regulatory mutations in control regions adjacent to structural genes which lead to reduced activity are much more difficult to recognise as they cannot be readily distinguished from mutations within structural genes themselves. The *sB₉₀* mutation, however, is a clear indication that such mutations do exist.

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α and β Chains of the major haemoglobin and a note on the minor component of *Tarsius*

THE position of *Tarsius* (the tarsier) in the evolution of the primates has long been controversial. Gadow¹ placed it in a separate suborder, but Pocock² allied it with the monkeys, apes and man in the Haplorhini, assigning the lemurs, lorisooids and tree shrews to the Strepsirhini. This was accepted by Hill³ but Simpson⁴ divided the primates into two suborders, Anthropoidea and Prosimii, placing *Tarsius* in the latter together with lemurs, lorisooids and tree shrews, though as a separate infraorder. The problem arises

because of resemblances between *Tarsius* and the anthropoids. These include the absence of a rhinarium, a retinal structure similar to the anthropoid fovea and a haemochorial placenta, compared with the epitheliochorial form of prosimians, *Tarsius* is often regarded as a 'living fossil', implying that little evolution has taken place since its divergence from the other primates. The problem may be solved with the aid of data on the primary structure of mammalian proteins⁵: there is sufficient evidence about haemoglobin, for example, to construct a hypothetical ancestral primate sequence⁵⁻⁷. Using two samples of blood from *T. bancanus* collected during the past 3 yr, we have determined the α and β Hb chain sequences and found that, at the molecular level, *Tarsius* is not a 'living fossil' and that the most parsimonious globin trees cluster *Tarsius* with the anthropoids and not the prosimians, lending support to the Haplorhini-Strepsirhini classification^{2,3}.

Both our samples had two haemoglobins on starch-gel electrophoresis at pH 8.6 (Tris-EDTA-borate)⁸; the major and minor components had mobilities identical with those of human HbA ($\alpha_2\beta_2$) and HbA₂ ($\alpha_2\delta_2$) respectively. The two components were separated by ion exchange chromatography on DEAE-Sephadex⁹ and absorbances at 540 nm of the pooled fractions showed the minor component to be 18% of the total haemoglobin in each case. The major component was used for sequence studies. Globin was prepared by acid-acetone precipitation¹⁰ and the chains were separated¹¹. The amino acid sequences of the α and β chains were determined after tryptic digestion of the aminoethylated chains. The evidence for the sequences will be given in more detail elsewhere.

The amino acid sequence of the tarsier α chain differed from the human α chain in eleven positions and the β chain had fifteen differences from the corresponding human chain. Adequate evidence was obtained for both sequences except for two short regions which are based on amino acid compositions and homology with other globin chains. These are residues 129-136 of the α chain (from a chymotryptic peptide) and residues 67-75 of the β chain, the sequence of which was postulated after amino acid analysis of two thermolytic peptides.

The available data on primate α and β chains¹²⁻²¹ is summarised in Tables 1 and 2 respectively. Only residues that are variable among primates are shown together with the hypothetical primate and anthropoid ancestral residues constructed by Goodman *et al.*⁷ using the parsimony principle. The construction of these ancestral residues was not biased by the data for the tarsier. Only sequences for which there is a reasonable amount of evidence have been considered.

There are several changes from the ancestral sequence in both chains which, among primates, are unique to the tarsier (α 15, 53, 68, 73, 129; β 6, 19, 21, 52, 58, 69, 73, 75) and certainly do not give the impression that tarsier is a

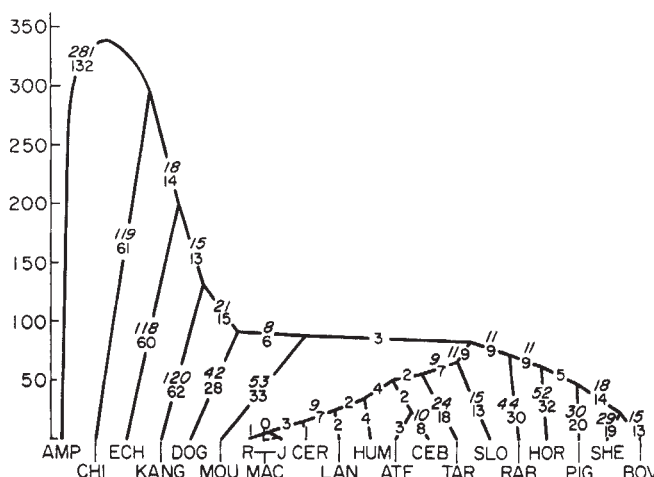


Fig. 1 Parsimony tree requiring 664 nucleotide replacements for 20 taxa on using a combined α - and β -globin alignment. Link lengths are the numbers of nucleotide replacements between adjacent ancestral and descendant sequences; italicised numbers are link lengths corrected for superimposed replacements by the augmentation algorithm of Moore and Goodman^{6,7}. The ordinate scale, in millions of years, is inferred from fossil evidence on ancestral splitting times of the taxa represented by the sequences. The taxa are: AMP (amphibian, represented by newt α and frog β), CHI (chicken), ECH (echidna), KANG (kangaroo), DOG (dog), MOU (mouse), R-MAC (rhesus macaque), J-MAC (Japanese macaque), CER (*Cercopithecus*), LAN (langur or *Presbytis*), HUM (human), ATE (*Ateles*), CEB (*Cebus*), TAR (*Tarsius*), SLO (slow loris or *Nycticebus*), RAB (rabbit), HOR (horse), PIG (pig), SHE (sheep), and BOV (bovine). The α - and β -globin sequences from these animals are referenced in Beard and Goodman²⁶.

'living fossil' at the molecular level. Most of the residues at which the tarsier sequences are unique among primates do not have a defined functional role^{22,23}. One change in the α chain, a Val instead of Leu at position 129 (H12), or position 136 (H19), is at the α -haem contact. In the β chain, position 75 (E19) is internal and helps stabilise the tertiary structure. The Met found in tarsier has also been found in sheep β chain.

The original aim of the work on tarsier haemoglobin was to acquire sequence information relevant to the animal's phylogenetic position. If the ancestral primate sequence is correct it may be possible, by examining the known sequences, to find changes common only to tarsier and prosimians or to tarsier and the anthropoids. But if tarsier diverged from the anthropoid branch very soon after the prosimians, there may not have been time for substitutions to occur which would be inherited by both lineages. For the discussion which follows only those globins listed in Tables 1 and 2 have been considered.

Table 1 Variable residues in primate α -globin chains

Residue no.	8	12	15	19	21	23	53	57	67	68	71	73	78	111	113	116	129
Primate ancestor*	Thr	Ala	Gly	Gly	Ala	Asp	Ala	Ala	Thr	Asn	Ala	Val	Ser	Ser	His	Asp	Leu
Anthropoid ancestor*	—	—	—	—	—	—	—	Gly	—	—	—	—	Asn	Ala	—	—	—
<i>Nycticebus</i>	—	—	Glu	Ser	—	—	—	—	—	—	Ser	—	—	Cys	—	—	—
<i>Tarsius</i>	—	—	Asp	—	—	—	Ser	Gly	—	Thr	Gly	Ile	Asn	Cys	—	—	Val
<i>Ateles</i>	Ser	—	—	—	—	—	—	Gly	—	—	—	—	Asn	Ala	—	—	—
<i>Cebus</i>	—	Thr	—	—	—	—	—	Gly	Ser	—	—	—	Asn	Ala	—	—	—
<i>Cercopithecus</i>	Ser	—	—	—	—	Glu	—	Gly	—	Leu	Gly	—	His	Ala	Leu	Glu	—
<i>M. mulatta</i>	Ser	—	—	—	—	Glu	—	Gly	—	Leu	Gly	—	Asn	Ala	Leu	Glu	—
<i>M. fuscata</i>	Ser	—	—	—	—	Glu	—	Gly	—	Leu	Gly	—	Asn	Ala	Leu	Glu	—
<i>Presbytis</i>	—	—	—	—	Gly	Glu	—	Gly	—	—	—	—	His	Ala	Leu	Glu	—
<i>Homo</i>	—	—	—	Ala	—	Glu	—	Gly	—	—	—	—	Asn	Ala	Leu	Glu	—

Residues that are variable among primates and differ from the primate ancestor are shown. The sequences considered are those of *Nycticebus coucang*¹², *Ateles geoffroyi*¹³, *Cebus apella*¹⁴, *Cercopithecus aethiops*¹⁵, *Macaca mulatta*¹⁶, *Macaca fuscata*¹⁷, *Presbytis entellus*¹⁸, and human (*Homo*)^{19,20} α globins.

* From the parsimony construction of Goodman *et al.*⁷ (provided by Professor M. Goodman); these constructions also include the following alternative parsimony solutions at positions 23 (primate Glu, anthropoid Glu) and 111 (Ala, Ala). With these alternatives, however, inclusion of the tarsier sequence would add more mutations to the tree than with the solution used above.

Table 2 Variable residues in primate β -globin chains

Residue no.	5	6	9	13	19	21	22	33	43	50	52	56	58	69	73	75	76	87	104	112	121	125	126	128	139
Primate ancestor	Ala	Glu	Ser	Ala	Asn	Asp	Glu	Val	Glu	Ser	Asp	Gly	Pro	Gly	Asp	Leu	Asn	Lys	Arg	Phe	Glu	Gln	Val	Ala	Asn
Anthropoid ancestor	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Cys	Val	—	—	—	—
<i>Nycticebus</i>	Gly	—	—	—	—	—	Asp	—	—	—	Ser	—	—	Ser	—	—	Thr	—	—	Cys	Asp	—	—	Ser	—
<i>Tarsius</i>	—	Asp	Ala	—	—	Glu	Asp	—	Asp	Thr	Ala	Ser	Ala	Asn	Glu	Met	Ala	Gln	—	Cys	—	—	Leu	—	Thr
<i>Ateles</i>	Gly	—	Ala	—	—	—	—	—	—	Thr	—	Asn	—	—	—	—	Thr	Gln	—	Cys	—	—	—	—	—
<i>Cebus</i>	—	—	—	Thr	—	—	—	—	—	Asp	Thr	—	—	—	—	—	Ala	Gln	Lys	Cys	—	—	—	—	—
<i>Cercopithecus</i>	Pro	—	Thr	Thr	—	—	—	—	—	—	—	—	—	—	—	—	Thr	Gln	Lys	Cys	—	—	—	—	—
<i>M. mulatta</i>	Pro	—	Asn	Thr	—	—	—	Leu	—	—	—	—	—	—	—	—	Ala	Gln	Lys	Cys	—	—	—	—	—
<i>M. fuscata</i>	Pro	—	Asn	Thr	—	—	—	—	—	—	—	—	—	—	—	—	Ala	Gln	Lys	Cys	—	—	—	—	—
<i>Presbytis</i>	Pro	—	Ala	—	—	—	—	—	—	—	—	—	—	—	—	—	Ala	Gln	Lys	Cys	—	—	—	—	—
<i>Homo</i>	Pro	—	—	—	—	—	—	—	—	Thr	—	—	—	—	—	—	Ala	Thr	—	Cys	—	Pro	—	—	—

Residues which are variable among primates and differ from the primate ancestor are shown. The sequences considered are those of *Nycticebus coucang*⁶, *Ateles geoffroyi*^{13,21}, *Cebus apella*¹⁴, *Cercopithecus aethiops*¹⁵, *Macaca mulatta*¹⁶, *Macaca fuscata*¹⁷, *Presbytis entellus*¹⁸, and human (*Homo*)¹⁹ β globins.

* From the parsimony construction of Goodman *et al.*⁷ (provided by Professor M. Goodman); these constructions also include the following alternative parsimony solutions at positions 9 (primate Ala, anthropoid Ala); 50 (Ser, Ser), 52 (Asn, Asp), 69 (Ala, Gly), 87 (Lys, Gln), 112 (Ile, Cys and Val, Cys). With any of these alternatives, however, inclusion of the tarsier sequence would add more mutations to the tree than the solution used except at β 9 and β 87 where the alternatives could be used equally well as the anthropoid ancestors.

Unfortunately this selection only includes one prosimian, *Nycticebus*, although several anthropoids are known. Sequences have been reported (as unpublished data^{24,25}) for certain other prosimians but adequate evidence has not been published.

In the α chains shown in Table 1 there have been several changes from the ancestral sequence which could provide useful information. There is one residue which only *Tarsius* and *Nycticebus* have in common among the primates, that is Cys at position 111 instead of the ancestral Ser. At position 15 there is also evidence for a common ancestor of *Tarsius* and *Nycticebus*. On the other hand, there are two positions at which *Nycticebus* retains the ancestral sequence while tarsier and most of the anthropoids share a common change (57—Ala to Gly; 78—Ser to Asn). Both are single-base changes.

If one considers the β globins in Table 2, there is one position (22) at which tarsier and *Nycticebus* share a common change from the ancestral sequence. Also at positions 52 and 69, fewer mutations need have occurred if they shared a common ancestor after diverging from the anthropoids. At position 112, however, a Cys is present in tarsier and the anthropoids instead of the ancestral Phe, while *Nycticebus* has a Val residue at this position. Also at positions 50 and 76 *Nycticebus* has retained the ancestral sequence while tarsier and several anthropoids have acquired the same change. Thus, with respect to both α and β -chain data, the hypothetical ancestral residues constructed independently of tarsier fail to indicate whether *Tarsius* is closer phylogenetically to Anthropoidea or to Prosimii.

Since the tarsier sequence might contain information needed for a closer approximation to the ancestral sequence, Beard and Goodman²⁶ included it in a combined α - and β -globin alignment and used a branch-swapping procedure⁷ to search extensively for the most parsimonious tree. In the several equally parsimonious alternatives found, tarsier was always closer to the Anthropoidea than to the slow loris, which supports the inclusion of the tarsier in a subdivision of the primates, Haplorhini^{2,3}. Of these trees, that in Fig. 1 conforms closely to traditional evidence on the phylogeny of placental mammals. But more prosimian species need to be included to test adequately the conclusion and only two additional mutations would be added to the path length of the tree if tarsier and slow loris still shared a common ancestor (as prosimians) after the divergence of the Anthropoidea from prosimian primates. On the other hand, six additional mutations would be added if the positions of tarsier and slow loris were reversed. Moreover, if the representation of tarsier's phylogenetic position in Fig. 1 is correct, it is notable that the ancestral haplorhine node is closer to the ancestral anthropoid node than to the ancestral primate node. It should be noted that tarsier diverges more from the haplorhine ancestor than do most of the Anthropoidea. This clearly supports the

impression (see above) that at the molecular level, the tarsier is no 'living fossil'.

Finally, we wish to add a note on the minor component of tarsier haemoglobin. Minor components with a δ chain instead of a β chain exist in man, the apes and New World monkeys but not Old World monkeys. Following the observation of a minor haemoglobin in tarsier with A₂-like mobility⁸ it has been suggested that a β protodelta duplication occurred in a tarsoid-like line ancestral to both *Tarsius* and Anthropoidea²⁷.

The δ chains in New World monkeys, apes and man all have Arg and Asn in positions 116 and 117 respectively instead of His at both these sites which occurs in the β chains. Gly, however, is present at position 5 in both chains of the New World monkeys (except for *Cebus* which was Ala in the β chain) whereas Pro is present in both chains of apes and man. These latter data suggest that more than one gene duplication has occurred^{21,28}.

Starch gel electrophoresis (in 6 M urea) indicated that the tarsier haemoglobins differed in their non- α chains. The amino acid compositions of those tryptic peptides containing the sites important in deciphering the origin of the δ chains of primates (positions 116, 117 and 5) were identical in both haemoglobins of tarsier. There is, therefore, no direct evidence that the β -like chain of the minor haemoglobin of *Tarsius* is orthologous with either man-ape or New World monkey δ chain. It should also be noted that the 'minor' component of tarsier is present in relatively high concentration (18% of total). In other species the concentration of minor component is between 0.5% and 6% (ref 21). It would be interesting to examine the haemolysates of other tarsier blood samples and look at the frequency of occurrence of a minor component in all species of tarsier. Two major haemoglobins have been observed in several prosimians^{8,29}.

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Thymine hydroperoxide as a mediator in ionising radiation mutagenesis

WE describe the mutagenic action of chemically synthesised *cis*-5,6-dihydro-6-hydroperoxy-5-hydroxythymine (6-TOOH)¹ on transforming DNA of *Haemophilus influenzae*^{2,3}, and suggest a mechanism by which ionising radiation induces mutations *in vivo*.

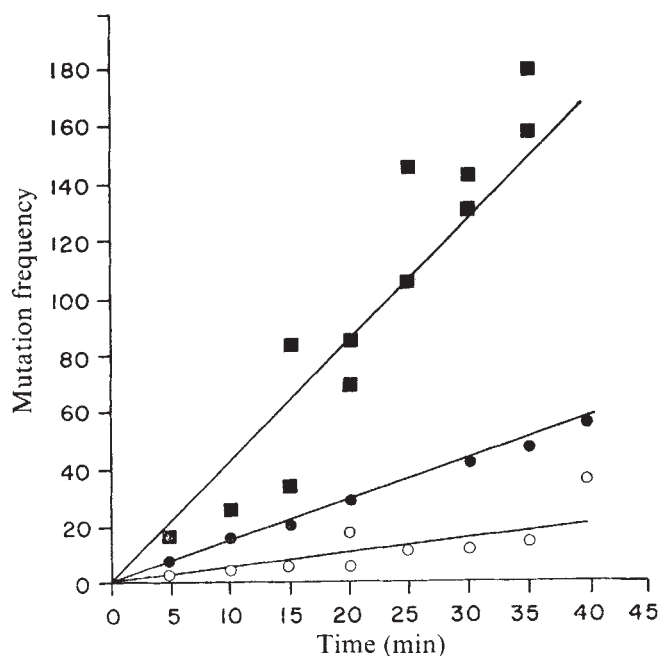


Fig. 1 Effect of 6-TOOH concentration on the induction of mutations in DNA. Weighed quantities of synthetic 6-TOOH¹ were mixed with solutions of *H. influenzae* DNA (10 µg ml⁻¹) carrying the marker high resistance to streptomycin (2,000 µg ml⁻¹). The solvent was 0.08 M Tris buffer, pH 7.8-0.04 M NaCl and 0.25 mM CuSO₄. Samples from the reaction mixture, held at 25 °C, were removed at intervals diluted and assayed with competent *H. influenzae*³ for markers carrying resistance of 8 µg ml⁻¹ of kanamycin and for the surviving streptomycin resistance. The mutation frequency is the ratio of the fraction of mutants (*M*) to the fraction of implicit marker (*S*); (*M*_{*t*}/*M*₀)/(*S*_{*t*}/*S*₀) where the values at time *t* are compared with that initially (0) present. The initial mutation frequency is unity and not zero as it might appear in the drawing. 6-TOOH concentration was: ○, 11.3 mM; ●, 22.6 mM; ■, 45 mM.

Since Muller⁴ discovered radiation mutagenesis no molecular mechanism to explain it has been established. Irradiation of aqueous solutions of biological materials in the presence of air generates various peroxides, including hydrogen peroxide, pyrimidine hydroperoxides⁵⁻⁷ and lipohydroperoxides⁸⁻¹⁰. Other organic peroxides¹¹⁻¹⁵ have been found to be mutagenic but they have not been identified in irradiated cells. Hydrogen peroxide is only weakly mutagenic for bacteria¹⁶ and not at all for transforming DNA¹⁵. The mutagenic potential of lipohydroperoxides has been indicated but not established¹⁷. Thymine hydroperoxide was reported to be mutagenic for *Escherichia coli*¹⁸, but this did not get support¹⁹ and later reviews^{20,21} did not mention it. Our work began with the finding that 6-TOOH, the agent used by earlier workers¹⁸, was mutagenic for *H. influenzae*. But a mechanism of action is not easily traced in an *in vivo* system, so the relatively uncomplicated transforming DNA freed of other cellular components was chosen for study. Tris-buffered solutions of purified *H. influenzae* transforming DNA³ were exposed to 6-TOOH in the presence of a transition metal ion. Changes in the transforming DNA³ leading to mutations were recognised as new resistance to either of two antibiotics which the DNA conferred on competent *H. influenzae* that are normally sensitive to the antibiotics. Before treatment with hydroperoxide, the transforming

Table 1 Effect of metal ions on mutagenic action of 6-TOOH

Metal ion	Δ Mutation frequency per min
Cu ²⁺	1.3
Co ²⁺	0.35
Mn ²⁺	0.1-0.3
Fe ²⁺	0.2
Zn ²⁺	0.2
None	0.08
Ca ²⁺	0.08
Mg ²⁺	0.08
Cu ²⁺ + 10 ⁻³ M EDTA	<0.02

The conditions of this comparison were those described in Fig. 1 except that the nature of the metal ion was varied. The initial concentrations of 6-TOOH was 22.6 mM and the metal ion was 0.25 mM in all instances.

DNA conferred resistance to streptomycin, a property which was followed to evaluate the destructive action of the hydroperoxide of genetic markers. Knowledge of this destructive action is necessary if the mutation frequency is to be calculated.

The mutagenic action of 6-TOOH in the presence of Cu²⁺ is shown in the data plotted in Fig. 1. The frequency of colonies resistant to kanamycin increased linearly with time of exposure to 6-TOOH and the rate was roughly proportional to the hydroperoxide concentration. A comparable increase in frequency of resistance to novobiocin was also found. In very similar experiments, the effect of different transition metal ions on 6-TOOH mutagenesis was determined. The results are shown in Table 1. Clearly among the metal ions examined, Cu²⁺ is much more effective than Co²⁺, Mn²⁺, Fe²⁺ or Zn²⁺ and two non-transition metal ions Ca²⁺ and Mg²⁺ which showed no mutagenic action. The level of metal ion represents that which was introduced and not the level of free ionic form during the reaction. The latter was almost certainly lower, for metal ions readily complex with constituents of the system to form weakly dissociated units. The dependence of 6-TOOH mutagenesis on the concentration of metal ion is seen in the results in Fig. 2. The rate of mutagenesis seems to be roughly a direct function of the Cu²⁺ concentration. When EDTA was added, mutagenesis by the 6-TOOH was completely blocked. The weak, but definite, mutagenesis observed when no metal ion was added may

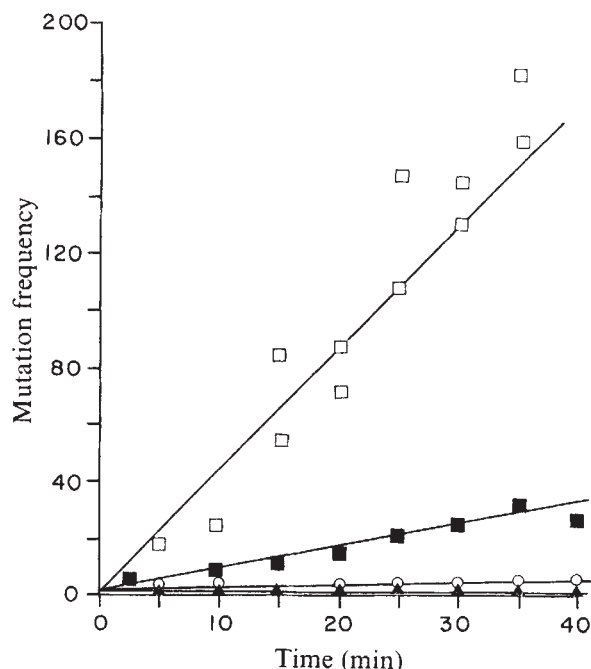


Fig. 2 Effect of copper ion concentration on the generation of mutations in DNA by 6-TOOH. The conditions were the same as described in Fig. 1 except that the 6-TOOH concentration was held at 45 mM and the Cu^{2+} was varied. ○, No added Cu^{2+} ; ■, 0.1 mM Cu^{2+} ; □, 0.25 mM Cu^{2+} ; ▲, 0.5 mM Cu^{2+} + 1 mM EDTA.

have been due to trace quantities of metal ions introduced with the DNA or the inorganic salts. Taken altogether, these results suggest that 6-TOOH in cooperation with certain metal ions, produces changes in transforming DNA that lead to mutations, although a detailed mechanism remains to be established. The involvement of transition metal ions and peroxides, which had been recognised earlier^{15,19}, probably means that free radicals are involved^{22,23}. Comparable mutagenic action of 6-TOOH on denatured and native transforming DNA seems to eliminate consideration of the denaturing effect of Cu^{2+} on DNA^{24,26}. In the absence of metal ion, 6-TOOH produced changes in bases guanine, cytosine, and thymine²⁷.

Pyrimidine hydroperoxides formed in DNA would be in close proximity to other bases and so might be highly mutagenic as a result of interaction, but neither these *in situ* hydroperoxides nor their glycol products of reaction would be planar and probably could not pair with other bases²⁸. Excision of the pyrimidine hydroperoxides from DNA^{29,30} *in vivo* would not be expected to reduce their mutagenic action below the level shown in the study reported here.

Hydrogen peroxide in the presence of Cu^{2+} failed to produce mutations in *Haemophilus* transforming DNA, which indicates that H_2O_2 is not an active intermediate responsible for the mutagenic action of 6-TOOH. This was supported by the finding that catalase, which destroys H_2O_2 , did not reduce the mutagenic power of 6-TOOH. Absolute increases in the mutant frequency in early samples of 6-TOOH mutagenesis of transforming DNA disposes of the argument that increases in the mutant frequency may be due to a selective advantage caused by a higher resistance of the mutants to the lethal action of the hydroperoxide. Studies (to be published) of the action of 6-TOOH on Ames' histidine mutants of *Salmonella typhimurium*³¹ indicate that the type mutants produced by this agent are substitution rather than frameshift. In this respect, the action of 6-TOOH is similar to that of alkylating agents²⁸.

Based on our results, the following mechanism for *in vivo* radiation mutagenesis is proposed, as a working hypothesis. Ionising radiation produces hydroperoxides of pyrimidines or their derivatives in the DNA or metabolic pool of cells. These hydroperoxides interact with transition metal ions forming free radicals which react with and modify neighbouring or other bases of the cell's genome. Some of these modified bases cause base mispairing during replication leading to what are generally regarded as mutations.

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matters arising

Clocklike behaviour of biological clocks

IN a recent review article¹, Winfree discussed a number of mechanisms that have been proposed to explain the dynamical nature of circadian clocks. The statement, "limit cycle excluded experimentally", appears as the heading of a section which proceeds to propose alternative mechanisms. Although the statements in the text qualify the experimental conclusions as excluding only a certain type of limit cycle, the rather sweeping statement in the heading is the one which is more likely to impress the mathematically unsophisticated reader. For this reason, we would like to emphasise some of the alternative mechanisms.

The data on which this argument is based result from two-pulse experiments². After a relatively weak light pulse which perturbs the system, a second, much stronger one is applied. Because the *Drosophila pseudoobscura* eclosion rhythm can be monitored only for ten cycles or less, the second pulse follows the first by no more than 48 h: two cycles. It is found that the closer the first pulse brings the system to the hypothesised singularity, the flatter is the resulting, second-pulse resetting curve. The important point is that this flattening could be explained by a contraction in the net amplitude displayed during the two recovery cycles.

Winfree suggests two hypotheses to explain such a reduction. (1) A limit cycle process is not operative; rather, a single master oscillator displays conservative dynamics thereby allowing permanent amplitude reduction. (2) A limit cycle process is operative in a population of essentially uncoupled oscillators whose combined action gates the observed rhythm. Dephasing within the population will reduce the net amplitude displayed. Our aim is to suggest that other possibilities exist between these two extremes.

With regard to the first, Winfree has shown that recovery to the limit cycle most probably does not occur within two cycles. He proceeds, however, as though recovery never occurs, to exclude the possibility of a limit cycle model. It is easy to demonstrate that the two-pulse experiment data are compatible with a slowly recovering model. Figure 1 shows a phase plane plot

produced by computer simulation of one of the earlier models^{3,4}. This particular plot shows the trajectory of a system starting very near the singularity. Strong pulses given to the system within the first two recovery cycles would result in flatter resetting curves the closer the initial pulse approaches the singularity. (Roughly speaking, the range of the resetting curve corresponds to the projection of a "cycle" on the S axis.) Similar responses can result from any system satisfying a set of rather general conditions³.

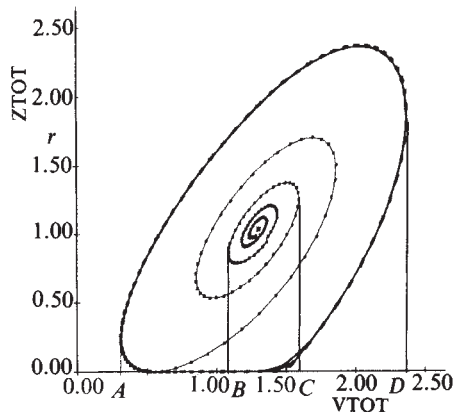


Fig. 1 CALCOMP plot of a trajectory starting near the singularity for the system described by differential equations:

$$\begin{aligned} dr/dt &= r - 0.8s - 0.3s^2 + 0.5 & r \geq 0 \\ ds/dt &= r - 0.8s \end{aligned}$$

This has been proposed as a model for circadian clocks^{3,4}. Strong light pulses move r to zero so that the resetting zone, when the system is on the limit cycle, is AD . Two days away from the postulated initial condition, not far from the singularity, will be small, BC .

The gradual recovery from the singularity to the limit cycle is actually a property shared by many of the proposed models of circadian rhythms as well as other bio-oscillators⁵⁻⁸. Higgins has published a number of phase plots similar to our Fig. 1 (ref. 8). Wever has also described similar phenomena⁹. Quick recovery (that is recovery within a day) to the limit cycle has been the feature of some of the earliest models only and it was the result of an

attempt to simulate the effects of strong pulses.

Many other interesting possibilities occur if one uses models involving populations of coupled oscillators. Such models are quite popular^{4,9-11} and have been successful in simulating phenomena such as frequency doubling (rhythm splitting)^{4,9-11}. In a population of strongly coupled oscillators, each of which in isolation quickly recovers its limit cycle, a return to the original trajectory may be slowed by resynchronisation among the various units. Consider in addition, a population of units with a stable limit cycle surrounding both an antilimit cycle and stable singularity. If the units are not exactly identical, they will form a "cloud" along the limit cycle. Then the first pulse may send some units into the singularity's region of attraction while leaving others in the region of attraction of the limit cycle. Coupling between units would prohibit a quick return to the respective limit sets (cycle or singularity). Thus an intermediate trajectory will be followed, possibly with a split in the population in which one group resides near the singularity, the other near the limit cycle. A second strong pulse would drive both groups towards the limit cycle where mutual synchronisation would reform the original cloud. The closer the initial push to the singularity, the more units approach that point, and, in the limit, all units reside there indefinitely. In that case, a perfectly flat resetting curve results after the second pulse. (Amplitude was abolished.)

The number of models simulating two-pulse experiment results is certainly quite large and the choice among them may have to be made on the basis of other factors. We prefer limit cycle models (possibly involving strongly coupled oscillators) because they seem compatible not only with the data described in the literature on circadian rhythms^{4,11} (including the two-pulse experiments), but also with other systems such as the metabolic oscillations in yeast, where behaviour similar to that of *D. pseudoobscura* is observed^{12,13}. The yeasts are known to mutually synchronise within a cycle¹⁴ and apparently are governed by limit cycle dynamics^{8,15}. Furthermore, dynamical systems displaying conservative oscillations are structurally un-

stable in the extreme¹⁶ and isoperiodic only in the most unusual conditions^{4,17}.

In conclusion, we would like to emphasise that Winfree's elegant experiments have revealed certain aspects of the dynamic behaviour of the circadian regulator of the *Drosophila* eclosion rhythm and represent a prime example of the use of mathematical models for understanding the nature of biological mechanisms. It is only unfortunate that the paper chose to emphasise especially in its title and section titles only two of the many hypotheses still available. Furthermore, most of these hypotheses are very "clocklike" as long as the term "clock" is used to mean a general time-keeping mechanism.

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WINFREE REPLIES—There seem to be three issues, all resolvable. First, Aldridge and Pavlidis¹ rightly observe that my experiments² exclude only "quickly recovering" limit cycle models, by virtue of showing no change in a measure of the amplitude of a circadian oscillator during 48 h after each of 30 different adjustments of that amplitude. "No change" means roughly "less than $\pm 10\%$ ". They rightly object that my adjective "quickly recovering", used everywhere else, was deleted from a section heading which then read "limit cycle excluded experimentally"—whereas a very slow recovery of amplitude remains unexcluded, as does a very slow decay of amplitude.

On the other hand, according to my data, the spiralling of trajectories in

the descriptive metaphor of their Fig. 1 is definitely too fast: almost all trajectories strike arc *AD* within 48 h, ensuring complete recovery to a standard cycle, contrary to observations.

Second, Aldridge and Pavlidis agree that my experiments suggest a long-lasting heterogeneity of state among two-to-many similar circadian mechanisms with each animal. I chose to emphasise phase incoherence and that this could derive from relative independence of these autonomous (possibly cellular) mechanisms. Assuming such independence and persistent incoherence in a population, the metaphor of Fig. 1 (among others) is perfectly compatible with the emphasised peculiarities of the circadian rhythm of *Drosophila*. This was intended to be the main point of my "Unclocklike . . ." paper in *Nature*³. Aldridge and Pavlidis chose to emphasise an alternative possibility, that some oscillators in the population are switched off, the others remaining synchronous. This model abandons Fig. 1 for a more complicated drawing with two limit cycles (one unstable) and supposes rapidly synchronising interactions among cells. I agree that this is a sensible alternative, which I overlooked. When a histological assay of circadian state becomes available, the two models—one predicting phase heterogeneity and independence, one predicting amplitude heterogeneity and coupling—should be clearly discriminable.

The third point is terminological. It is usual for familiar words, adopted into a specialised area, to change their connotation as perspectives change and scholars make more refined distinctions. As adopted in the 1950s "clock" connoted little more than adaptive stability of period. But by the mid-1960s much of the literature makes sense only if a more restrictive implicit connotation is recognised, namely what is distinguished in the cell-cycle literature⁴ as a "simple clock". This is a mechanism which, like a commercial clock or a music box, unlike a dynamical oscillator, can change only its phase, having no states off a unique causal cycle. In pointing to the singularity and to amplitude lability as two "unclocklike" features of the circadian mechanism, I have adhered to this more restrictive usage, distinguishing "clocks" from "dynamical oscillators". In contrast, Aldridge and Pavlidis apparently use "clock" to mean "dynamical oscillator" as opposed to such alternative periodic mechanisms as a sequential state machine with one fixed cycle. Thus the "clocklike" features of their paper¹ are the same as the "unclocklike" features of mine³! I notice that Pittendrigh has simply abandoned the word in entitling a

current preprint ". . . circadian pace-makers".

I am delighted to have this critical exchange, and wish there were a lot more of it in the circadian literature.

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- ¹ Aldridge, J. F., and Pavlidis, T., *Nature*, **259**, 343–344 (1976).
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Structure of the galactic magnetic field

RECENTLY Somogyi¹ has presented arguments indicating that the power spectrum of the turbulent galactic magnetic field is of the form $k^{-\alpha}$, where k is the wave-number and $\alpha = 1.0$ – 1.8 depending on the model of cosmic-ray transport assumed. This spectrum is relevant for scales of 0.001–1 pc, and was obtained by noting the dependence of the cosmic-ray anisotropy and pathlength on energy. I wish to show that a similar conclusion follows from a related but independent argument based solely on the average cosmic-ray pathlength and observed properties of the large scale galactic magnetic field².

Consider a simple one-dimensional model for the diffusion of cosmic rays perpendicular to the plane of the Galaxy, with constant diffusion coefficient $K_{\perp}$ and free escape at the boundaries $z = +L$ and $z = -L$. For observers near the galactic disk ($z = 0$) it is straightforward to show that the mean path λ is given by

$$\lambda = nm_{\text{H}}c\langle t \rangle = (5/12)nm_{\text{H}}cL^2/K_{\perp} \quad (1)$$

where n is the interstellar density of hydrogen, m_{H} is the mass of a hydrogen atom, c is the speed of light, and $\langle t \rangle$ is the average cosmic-ray lifetime^{2,3}. From equation (1), one may infer a value of $K_{\perp}$, given the other parameters, and for those listed in Table 1, the value of $K_{\perp}$ varies from 1.2×10^{26} – 1.2×10^{28} cm² s⁻¹, with a nominal value of $\sim 10^{27}$ cm² s⁻¹. This value corresponds to the 'observed' value of the cosmic-ray diffusion coefficient for those particles (with energies ~ 5 GeV per nucleon) which are responsible for spallation.

Next consider the derivation of the diffusion coefficient from the properties of the random interstellar magnetic field. Using standard diffusion theory^{4,5}, in the limit that the gyroradius r_g of the particle is much smaller than the magnetic-field correlation length l_0 , one obtains^{2,6}

$$K_{\perp} = F(\alpha)cl_0(r_g/l_0)^{2-\alpha} \quad (2)$$

Here $F(\alpha)$ is a factor of order unity², and it has been assumed that the magnetic-field power spectrum is flat for wave

numbers $k < (l_0)^{-1}$, and is proportional to $k^{-\alpha}$ for larger wave numbers. Jokipii and Lerche⁷ give $l_0 = 150$ pc, and find an average interstellar field of 3×10^{-6} gauss, so for ~ 5 GeV per nucleon particles, $r_g/l_0 \sim 1.4 \times 10^{-8}$. Because this ratio is so small, K_1 as determined from equation

Table 1 Adopted parameters

Parameter	Nominal value	Range
n	1 cm^{-3}	0.2–2
L	$1.5 \times 10^3 \text{ pc}$	1.0–3.0
λ	5 g cm^{-2}	3–8
l_0	$1.5 \times 10^3 \text{ pc}$	0.5–2.0
B_0	$3 \times 10^{-6} \text{ gauss}$	2–5

(2) is very sensitive to the exponent α of the magnetic-field power spectrum. The values calculated from equation (2) are given in Table 2 for several values of α . The uncertainties given in Table 2 are obtained by varying the parameters through the range shown in Table 1. Comparing the 'observed' result from equation (1) with the entries in the table, one concludes that

$$\alpha = 1.5 \pm 0.2 \quad (3)$$

if the simple diffusive model of cosmic-ray transport is appropriate. The uncertainty quoted in equation (3) is the worst-case limit; the statistical uncertainty would be much less.

This value of the power spectral index ($\alpha \sim 1.5$) of the galactic magnetic

Table 2 Calculated diffusion coefficients

α	$K_1 (\text{cm}^2 \text{ s}^{-1})$
1.2	$(5.3 \pm 3.0) \times 10^{24}$
1.4	$(1.5 \pm 0.8) \times 10^{26}$
1.5	$(1.0 \pm 0.5) \times 10^{27}$
1.6	$(6.9 \pm 4.0) \times 10^{27}$
1.8	$(4.6 \pm 3.0) \times 10^{28}$

field is the same as the exponent of the interplanetary field and close to the Kolmogorov value of 5/3. The argument here includes the assumption of one continuous power law from the correlation scale of the fluctuations (150 pc) all the way down to the resonant wavelength for 5 GeV per nucleon particles (2×10^{-6} pc). For comparison, Somogyi obtains $\alpha = 1.7 \pm 0.1$ in the simple diffusion model for cosmic-ray transport with the distance to the boundary of the diffusing region independent of energy. His result is based on the resonant wavelengths for 10^{12} – 10^{15} GeV per nucleon particles, or scales of 4×10^{-4} – 0.4 pc.

Somogyi's result and that of equation (3) can be shown to agree even more

closely if one adopts his model and changes his parameters slightly. If the energy dependence of the cosmic-ray pathlength $\lambda(E) \sim E^{-\beta}$, with $\beta = 0.50 \pm 0.1$, as recent calculations indicate⁸, rather than $\beta = 0.2 \pm 0.1$ as adopted by Somogyi, then his results indicate that the size of the confinement volume does not depend on energy, and that $\alpha = 1.55 \pm 0.1$, in agreement with equation (3). Since equations (1) to (3) yield $\beta = 2 - \alpha$, the calculations in this letter predict $\beta \sim 0.5$, in agreement with the conclusions of Juliusson *et al.*⁸.

Thus the method presented here gives the result $\alpha = 1.5 \pm 0.2$, and predicts that the cosmic-ray pathlength varies as E^{-1} . Adopting this dependence of pathlength on energy, as supported by recent observations, one can use Somogyi's results to obtain $\alpha = 1.55 \pm 0.1$ and to show that the size of the diffusing region is independent of energy. The similarity of these two results, based on quite different assumptions, suggests that cosmic rays may be a useful tool for probing the interstellar magnetic field.

I thank Dr W. R. Webber for calling my attention to Somogyi's paper, and for his hospitality while this letter was being written. This work was supported by the Research Corporation through a Cottrell College Science Grant.

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SOMOGYI REPLIES—The value of α as derived by Owens¹ is based on a single point of the function expressed by his equation (2), and Owens assumed that the magnetic power spectrum had the form of a power function with a constant exponent in the range $2 \times 10^{-6} < k^{-1} < 150$ pc. In my paper², indications are given that the anisotropy and lifetime are power functions of energy, and it is proved that in this case the magnetic power spectrum is a power function at least in the range $10^{-3} \text{ pc} < k^{-1} < 1 \text{ pc}$ corresponding to the energy range $10^{12} \text{ eV} < E < 10^{15} \text{ eV}$ of anisotropy measurements with large statistical accuracies. The range of k as given in my paper² is thus an experimentally established one.

It would be difficult to argue which of the two α values is better established. Both are rather uncertain. I agree with

Owens that their agreement is remarkable, especially if recognising that they are based on different experimental evidences independent of each other.

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¹ Owens, A. J., *Nature*, **259**, 344–345 (1976).

² Somogyi, A. J., *Nature*, **225**, 689–690 (1975).

How specific are nuclear 'receptors' for thyroid hormones?

TATA has questioned¹ the biological relevance of the binding of T_3 to the nucleus. He presents four main arguments against a physiological role for the T_3 binding components in the nucleus. First, the presence of high-affinity, saturable binding sites for thyroid hormones of similar characteristics in a number of other subcellular fractions. Second, the lack of analogy with steroid hormone receptors. Third, the absence of parallelism between the binding of thyroid hormone analogues to the nucleus and the biological activity. Fourth, the fact that only 15–20% of the intracellular triiodo-L-thyronine is located in the nucleus.

We believe that several of Tata's conclusions are invalid.

On the first point. The K_a of the binding of T_3 by the nucleus has been incorrectly cited from the work of Samuels and Tsai² (Tata's ref. 19). The correct value is $3.3 \times 10^{10} \text{ mol}^{-1}$ ($K_d = 29 \text{ pmol}$) for the nuclei of GH₁ cells. The same high K_a has been found³ for the binding of T_3 to the nucleus of human lymphocytes and rat liver and kidney nuclei⁴. The fact that these values are much higher than those reported by Tata for the other subcellular components favours the possibility of a physiological role for this type of binding site within the nucleus.

With the technique used by Tata to measure specific binding (his Fig. 2) it is impossible to show high affinity binding sites in the nucleus, because the tracer concentration used is 60 times the maximal binding capacity in the incubation mixtures (binding capacity $2.9 \text{ fmol } T_3 \text{ per } 100 \mu\text{g DNA}$; see ref. 4).

Concerning the second point, the lack of analogy with steroid hormone receptor binding has been reported by Surks *et al.*⁵ (Tata's ref. 17) and Visser *et al.*⁶. Why does this lack of analogy imply that thyroid hormone binding in the nucleus is without physiological relevance? Why should the nuclear binding of chemically different substances proceed along identical lines?

The *in vitro* binding of T_3 to the nucleus diminishes when cytosol is added, because the cytosol competes for the same fixed amount of hormone in the incubation mixtures¹. But, *in vivo* that is not the case since both cytosol binding proteins and nuclear binding sites are in equilibrium with the same free hormone concentration outside the cell. This implies that the presence of more or less binding sites outside the nucleus probably has no effect on the degree of saturation of the nuclear binding sites with T_3 .

As for the third point, the parallelism between the nuclear binding of thyroid hormone analogues and hormonal activity has been shown by Oppenheimer *et al.*⁶ (not cited by Tata). Only Triac may be an exception. The completely different manners of binding of thyroid hormone analogues with cytosol and the nucleus^{4,7,8}, which implies a non-parallelism with biological activity, also suggests a physiological role for the T_3 binding components of the nucleus.

Finally Tata's fourth point presents no argument at all. For instance, does the fact that more than 60% of thyroxine in the human body is outside the cells imply that this compound has no intracellular action? On the contrary, it implies that the cell has a constant supply of the hormone, independent of variations in production. Perhaps a large binding capacity of T_3 outside the nucleus serves a similar purpose in the supply of T_3 to the nucleus.

We think that the arguments used by Tata are insufficient to reject the postulate that the binding of thyroid hormones to the nucleus is of physiological importance.

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my original article¹. Nonetheless they do not in any way modify the essence of my arguments and conclusions, which Docter *et al.*² have misread.

The absolute value of K_a for the interaction between the isolated nucleus and thyroid hormone is determined by the experimental conditions and is not relevant to my finding that in identical conditions all the subcellular fractions of rat liver exhibit rather similar T_3 -binding constants and properties. Unless similar experiments comparing nuclei with extranuclear fractions are performed by others, I do not see how K_a values of the order of $3 \times 10^{10} \text{ mol}^{-1}$ for isolated nuclei establish 'specificity'. Sterling and Milch have observed³ K_a values of 10^{11} mol^{-1} for the binding of T_3 to mitochondrial extracts. Unpublished data from my own laboratory reveal that concentrations of T_3 lower than those reported in my article did not appreciably alter the result I reported there¹. A study based on electron microscope autoradiography has also disclosed an ubiquitous intracellular distribution of thyroxine⁴. I agree with Docter *et al.*

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

that it is important to consider the situation *in vivo*, but their reasoning (their second point) can equally well be reversed: that is, the number of T_3 -binding sites within the nucleus may have little effect *in vivo* on the saturation of extranuclear sites.

As regards the parallelism between the relative binding affinities and physiological potencies of different hormone analogues (their third point), it would be desirable to observe it in any binding or receptor system. But such an analogy *per se* is of limited value since it is also observed in binding to inert materials like glass, paper, talc, and so on^{5,6}. All of these con-

siderations only highlight the difficulties of extrapolating from the results of interaction between isolated subcellular preparations and hormone 'receptors' *in vivo* of physiological significance.

Finally, as perhaps the first person to have pointed out the importance of the response of the target cell's nucleus to thyroid hormones, I would not like to rule out the possible location of hormone 'receptors' in that organelle. What I have, in fact, emphasised in my article¹ is the necessity for a more critical assessment of the current approaches based on the binding of thyroid hormones to isolated nuclei. As, and when, evidence is presented that such binding is related to a primary biochemical event leading to the physiological action of the hormone, then I shall be only too pleased to acknowledge that I am wrong in suggesting this note of caution and necessity for fresh thinking. Meanwhile, the past 50 years' history of thyroid hormone action has taught me not to be too dogmatic or 'fashionable'.

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¹ Tata, J. R., *Nature*, **257**, 18–23 (1975).

² Docter, R., Visser, T. J., and Hennemann, G., *Nature*, **259**, 345–346 (1976).

³ Sterling, K., and Milch, P. O., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3225–3229 (1975).

⁴ Manuelidis, L., *Yale J. biol. Med.*, **45**, 501–518 (1972).

⁵ Tata, J. R., *Rec. Progr. Horm. Res.*, **18**, 221–259 (1962).

⁶ Cuatrecasas, P., and Hollenberg, M. D., *Biochem. biophys. Res. Commun.*, **62**, 31–41 (1975).

Corrigendum

In the article "How specific are nuclear 'receptors' for thyroid hormones" by J. R. Tata (*Nature*, **257**, 18; 1975) the following corrections should be made.

On page 21, lines 22–26, the sentence should read . . . This is in agreement with recent reports from Baxter's laboratory^{23,40}, but whether or not the endogenous hormone-receptor complex is directly bound to DNA as concluded by these workers or merely to non-histone protein in intact chromatin, as proposed by others^{14,15,20–22}, is difficult to decide . . .

In Table 3 (page 22), the following corrections should be made:

The K_a (M^{-1}) value for rat pituitary tumour cell line nuclei should read 3.3×10^{10} .

The no. of sites for rat liver and kidney cytosol should read 0.53 pmol mg^{-1} liver and 2.9 pmol mg^{-1} kidney, respectively.

References 28,29,30 should read 29,30,31.

¹ Tata, J. R., *Nature*, **257**, 18–23 (1975).

² Samuels, H. H., and Tsai, J. S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3488–3492 (1973).

³ Tsai, J. S., and Samuels, H. H., *J. clin. Endocr. Metab.*, **38**, 919–922 (1974).

⁴ Visser, T. J., Docter, R., Stinis, J. T., Bernard, B., and Hennemann, G., *Thyroid Hormone Metabolism* (edit. by Harland, W. A., and Orr, J. S.), 35–45 (Academic, London 1975).

⁵ Surks, M. I., Koerner, D., and Oppenheimer, J. H., *J. clin. Invest.*, **55**, 50–60 (1975).

⁶ Oppenheimer, J. H., Schwartz, H. L., Dillman, W., and Surks, M. I., *Biochem. biophys. Res. Commun.*, **55**, 544–550 (1973).

⁷ Dillman, W., Surks, M. I., and Oppenheimer, J. H., *Endocrinology*, **95**, 492–498 (1974).

⁸ Koerner, D., Surks, M. I., and Oppenheimer, J. H., *J. clin. Endocr. Metab.*, **38**, 706–709 (1974).

⁹ MacLeod, K. M., and Baxter, J. D., *Biochem. biophys. Res. Commun.*, **62**, 577–583 (1975).

¹⁰ Charles, M. A., Ryffel, G. U., Obinata, M., McCarthy, B. J., and Baxter, J. D., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1787–1791 (1975).

TATA REPLIES—I apologise for the errors (see corrigendum, this page) in

reviews

The Evolution of IBP. (International Biological Programme 1.) Edited by E. B. Worthington. Pp. xx+268. (Cambridge University: Cambridge, London, New York and Melbourne, September, 1975.) £10.50. *Food Protein Sources.* (International Biological Programme 4.) Edited by N. W. Pirie. Pp. xx+260. (Cambridge University: Cambridge, London, New York and Melbourne, September 1975.) £7.50.

IN March 1959, shortly after the International Geophysical year ended, Sir Rudolph Peters conceived the idea of its biological equivalent. The idea gained the support of a number of biologists, although each had a different idea of what should be studied. In this way the International Biological Programme (IBP) was born. Through the efforts of the late Professor C. H. Waddington and Professor Guiseppe Montalenti, it had by 1964 grown into a decade long, seven-sided study of production ecology and human biology, entitled "The biological basis of productivity and human welfare." Now, nearly 20 years after its conception, the results are to be gathered in an 'international synthesis', a series of volumes of which Dr Worthington's kaleidoscopic study *The Evolution of IBP* is the first.

Dr Worthington was the scientific director of IBP, and the grasp of all its aspects which he demonstrates in this book, shows why Professor Waddington was so determined he should be appointed to that post. He modestly describes himself as editor of the book, although he is virtually its author and, as such, does an extremely workmanlike job of describing what IBP was. But for all its assiduous and dispassionate detail, Dr Worthington's book is not a history of IBP but a historical gazeteer, circumscribed by its own objectivity.

I suspect this has arisen paradoxically from Dr Worthington's deep involvement with the programme, for those so caught up in events are often the worst historians. Though Dr Worthington avoided producing an apologia for IBP he has not produced a critique either. He has simply chosen to record, rather than interpret, events. It is not that he sees history from the IBP's viewpoint: apart from IBP, he does not seem to see history at all. And so the events that have altered scientists' attitudes while IBP existed—events that must have moulded and been

moulded by IBP—are avoided, or only mentioned coyly. Dr Worthington's book is interesting and readable but IBP still needs its Margaret Gowing.

As does the topic to which Mr Pirie has addressed himself for the past 40 years—the need for increasing world protein production. Mr Pirie has long been the advocate of leaf protein as one method of bridging the world protein 'gap' and he persuaded IBP to stress leaf protein in one of its themes.

International biological programme

Unfortunately, over the years the protein gap has not so much been bridged as obliterated by systematic reductions in estimates of human protein requirements. Mr Pirie has not appreciated this alteration. Discussing the matter in 1969 he commented on the technical competence and professional integrity of some of those responsible for these estimates suggesting that they were biased by a desire to avoid change and a wish to resist wage demands based on the cost of food.

In this volume he avoids those issues and states simply that "more research is needed to meet the world's.....protein need than.....the equally important need for energy and vitamins". I do not believe it and suspect that Mr Pirie underestimates the inertia of his own ideas.

The definition of a protein source used in this book (at least 15% protein on a dry weight basis) is unfortunate, since it excludes the cereals, which provide over half the protein in most diets. Further complications are introduced by the exclusion of domesticated livestock and the inclusion of coconuts and minor seeds which contain less than 15% protein, but which are "interesting and liable to be overlooked".

The discussion of nutritive value is concentrated on protein value, and is confused by the use of terms like BV, NPU and PER without definition or details of the assay conditions. It is a pity that the IBP committee on units symbols and definitions which produced

the 50 page appendix to Dr Worthington's book omitted these. One suspects that some authors did not even consult IBP's own symposium on the evaluation of protein products.

Parts of the book are good—Sir Kenneth Blaxter on non-domesticated ruminants, for example—but on the whole it is a patchy compendium of some information on some aspects of some foods.

The power of the protein myth is such that its title assures it of a good sale, but I wish that Mr Pirie had devoted his talents and his time to a book on food and not fashion.

Fashion is perhaps the nub of it. It is inconceivable that an IBP started now would give such a high priority to protein. Nor that it would so easily dismiss Margaret Mead's plea for basing its Human Adaptability Programme on the social sciences. But then would a new IBP have such difficulty in getting a programme of production ecology started and funded?

IBP was overtaken by events, as anything started in 1959 had to be. In 1959 there was Eisenhower and Khrushchev. No one had heard of Yuri Gagarin, and very few of James Watson. Ecology was still about beetles.

IBP's operational decade encompassed Biafra and Vietnam. At its end there were famines in the Sahel and Ethiopia. A study of the biological bases of human welfare conceived now would be very different indeed: IBP seems academic, old fashioned and even slightly naïve.

Some of its synthesis volumes—the academic ones—will no doubt be standard texts, as some of its achievements are undeniable. Those like Mr Pirie's that are concerned so directly with human welfare run the risk of being outdated by the time they appear. Whether in the long run they'll be seen as the more significant or noble, remains to be seen.

John Rivers

Crop Genetic Resources for Today and Tomorrow. International Biological Programme 2.) Edited by O. H. Frankel and J. G. Hawkes. Pp. xiv+491. (Cambridge University: Cambridge, London, New York and Melbourne, September 1975.) £13.

IN recent years there has been an increasing awareness of the erosion of plant genetic resources due to the extension and intensification of land

use required to meet the demands of the human population for food and for manufactures. The concern that action should be taken to conserve these non-renewable resources has been displayed by a relatively few committed workers, among whom the editors of this book—Sir Otto Frankel and Professor Jack Hawkes—have been prominent. The institutional acceptance of the need for co-ordinated action first resulted in a joint review of the problem by the UN Food and Agriculture Organisation (FAO) and IBP. The outcome of this was the publication, as an IBP Handbook, of *Genetic Resources in Plants—their Exploration and Conservation* (edit. by O. M. Frankel and E. Bennett, Blackwell, 1970).

FAO continued to be active in the field of plant exploration and conservation through an Expert Panel. This led in 1974 to the establishment of an International Board for Plant Genetic Resources (IBPGR) under the auspices of the Consultative Group on International Agricultural Research which, co-ordinated by the World Bank, channels funds from governmental and non-governmental donors into the international research centres. The involvement of the IBPGR in plant exploration and conservation marks the initiation of a new phase in the development of this work and one in which it may be expected that wide scale effort will be backed by adequate resources.

Whatever happens in the new phase, however, it will be necessary for those concerned to draw to a large extent on the philosophies and methodologies developed during earlier work. These are well portrayed in *Crop Genetic Resources for Today and Tomorrow* which shows the point reached at the end both of the decade of the IBP and of joint work between FAO and IBP. The book contains contributions made at a technical conference organised by FAO and IBP, one of the important objectives of which was to produce a book collating a range of relevant subjects and approaches. It achieved this objective very well.

The subjects covered included the nature and basis of genetic variation in plant populations and how the explorer should sample populations and regions. Attention is paid to the ways in which, in crop plants, material collected from the wild or in primitive cultivars can be used to provide agriculturally valuable disease-, insect-, drought-, or frost-resistant parents in breeding programmes. As would be expected there is extensive discussion of the problems of how living plant material can be stored as seed, pollen, tissue or cells in culture, or as sporophytic plant members such as roots,

tubers or woody branches. Another approach to 'storage' is to protect plant populations *in situ*, and this is discussed in terms of population genetics by S. R. Jain. The storage of data relating to conserved plants, and their retrieval, is of crucial importance if the collections are to be used effectively by breeders; possible methods are discussed by D. J. Rogers and others.

This book is principally concerned with genetic conservation for plant-breeding purposes. The collectors and



Larger than life winged figure carrying a buck, probably Persian. Engraved by W. Holt from a relief in the Palace of Ashurnasirpal II at Nimrud. Taken from Fallow Deer: Their History, Distribution and Biology by D. and N. Chapman. Pp. 256. (Terence Dalton, Lavenham, Suffolk, UK, 1975.) £7.80. A whole chapter is devoted to Persian fallow deer, a very rare and endangered species.

the conservers are therefore undertaking a service for breeders. It would seem desirable on future occasions when the topic is discussed for consideration to be given to the methods by which genetic variability can be transferred in breeding programmes because this defines the systematic range that interests breeders and the kinds of genetic variation that may be useful.

There would have been increased stimulation in the book if the organisers of the conference had invited a devil's advocate to marshal the arguments against conservation instead of entirely confining themselves to the 'in group'. Good arguments can be advanced against the idea that we can always generate the required genotypes by mutation or by the release of variation following hybridisation but no opportunity was given for their expression. This would perhaps have caused

greater emphasis to be placed on the need to conserve not individual alleles but those complexes of genes that have become coadapted over long periods of selections. This was not ignored but was in my view not given the necessary weight.

I am also surprised that no attention was paid at the conference to the conservation of non-nuclear genes. We knew in 1970 that susceptibility to the 'T' race of *Helminthosporium* which caused serious maize losses in the US was determined cytoplasmically. The susceptibility is now known to be determined by mitochondrial DNA. There are important genes in both the mitochondria and the chloroplast DNA. This is well displayed by S. G. Wildman and his colleagues in relation to variation in the large subunit of Fraction 1 protein which is coded for in chloroplast DNA. In considering the kinds of resources we must conserve, and their selection and evaluation, it will be increasingly necessary in the future to give thought to organelles.

This book should provide a useful primer on crop plant collection, conservation and utilisation at a time when such is badly needed. **Ralph Riley**

Photosynthesis and Productivity in Different Environments. (International Biological Programme 3.) Edited by J. P. Cooper. Pp. xxiv+715. (Cambridge University: Cambridge, London, New York and Melbourne, September 1975.) £22.00.

THE main aim of IBP was the international study of the biological basis of productivity and human welfare and it is natural, therefore, that much of the research effort which IBP engendered should have centred on the primary stage of energy conversion, photosynthesis. The informed management of the world's resources and the support of its human population is dependent on a knowledge of this process in which solar energy becomes available for the nutrition of animals and man. Surprisingly little data was available on the productive potential of different natural and seminatural vegetation types until the initiation of the IBP in 1964 and much of the research which has since been conducted has been concerned with the accumulation of such vital information. Participants in this exercise gathered together in Aberystwyth in 1973 to present summaries of their findings and this book is based on a selection of the papers which were read at that Conference.

Perhaps the emphasis should be placed on the summary aspect of this collection of papers. One thing which has resulted from the decade of IBP is the assembly of vast quantities of

measurements of plant growth, taken both in the laboratory and the field, and it is obvious that no single volume could attempt to cover this material in any detail. This book does, however, set the scene for the more detailed reports, which have either preceded it or may now be anticipated. It is therefore aimed at the general biologist and presents a glimpse of the full spectrum of IBP work, but at the same time the fact that many papers contain previously unpublished results will make it appeal to research workers in the immediate field of productivity.

The first two parts of the book (out of a total of seven) are concerned with data accumulated on the primary production of terrestrial and aquatic ecosystems. Some of the individual papers within these parts are very broad, for example about 35 pages each on the productivity of forests, grazing lands and tundra, respectively. Such summaries must have been very difficult to write but more effort to avoid a parochial approach could have been made, especially by Kira, whose paper on forests is illustrated almost entirely by examples from Japan. The paper on grazing lands by Caldwell pays particular attention to the relative importance of the C4 pathway of photosynthesis in these habitats and provides a useful synthesis of this subject.

Although about a third of the earth's surface is technically arid (potential evapotranspiration exceeding precipitation), this is not reflected in the volume of literature relating to these situations. The inclusion of a chapter on primary production in deserts is therefore particularly welcome, especially the section dealing with the contribution of lichens to the energy input of such ecosystems.

If the surface area of the earth occupied by an ecosystem is the grounds for justifying its inclusion in this volume, then the agricultural ecosystems of the world certainly deserve a place. A chapter by Loomis and Gerakis is given over to this subject. They concentrate on crop adaptations and show how the photosynthetic characteristics for which selection has been made in domesticated plants varies with latitude. C4 species being little used in latitudes higher than 40°.

Aquatic ecosystems, both freshwater and marine, are discussed at some length and the contribution to productivity by macrophytes and microphytes is considered. The figure derived for the global production of marine microphytes in these chapters (31×10^9 t carbon yr^{-1}) is in close agreement with previously published figures.

The second half of the book turns from the study of whole ecosystems to more physiological aspects of the behaviour of light in plant canopies, photosynthetic studies of individual

species and the influence of environmental stresses upon photosynthesis and the use which is made of photosynthates.

Perhaps the most important contribution which this book makes is that it brings together reviews, most of which are extensive and well written, on a wide range of subjects from whole ecosystems, through whole plant physiology to subcellular biochemical ones. In doing this it may encourage workers in one field to become more aware of developments in related areas and to apply this knowledge to their own problems. Since the communication of ideas seems to be one of the greatest



Cocoa tree shown in bas relief on a Mayan stone tablet (1.3×1.3 cm) of the Late Classic Period (AD 600-900, from El Tajin, Veracruz, now at Instituto Nacional de Antropología e Historia Jalapa, Veracruz, Mexico. Taken from Diseases of Cocoa by C. A. Thorold. Pp. viii+423. (Clarendon: Oxford, 1975.) £11.00.

stumbling blocks to the general advancement of science, this book must be considered a valuable contribution. Indeed, if IBP had achieved no more than the bringing together of ecologists, physiologists and biochemists, then it would still have been worthwhile.

The price of this book precludes a very wide market, outside the better-endowed libraries, and this is unfortunate because many of the contributions would have been very useful to research workers and students alike.

P. D. Moore

Small Mammals: Their Productivity and Population Dynamics. (International Biological Programme 5.) Edited by F. B. Golley, K. Petrusewicz and L. Ryskowski. Pp. xxv+451. (Cambridge University: Cambridge, London, New York and Melbourne, September 1975.) £12.

NINE years of collaboration by small mammal biologists from twenty-seven countries led to this volume, number 5

in the IBP series. No-one, least of all the editors and contributors, would claim that all there is to be known about small mammals has been fully researched and documented but this does not mean that the present work has had too short a gestation period. Its birth is timely, its development has been satisfactory and its information content is something on which to reflect. For the first time we are provided, within the covers of one book, with an overview of developments in the global study of small mammals. Theory, practice, results and hypothesis, as related to small mammal productivity and population dynamics, all have their place.

The contributors seem to have experienced difficulty in defining the upper size limits of a small mammal but 5 to 6 kg is among the highest figures quoted; bobcats (*Lynx rufus*) can therefore be considered as representing a large 'small mammal' (chapter 8). Among the small mammals, however, most information derives from rodents and it is not surprising that much of the text is devoted to this group of animals. Needless to say gaps in our knowledge of small mammals are referred to on both a taxonomic (For example, bats) and geographical (For example, the tropics) scale.

The ecological implications of being a small mammal are highlighted in the introduction and the importance of these animals as consumers within natural and man-modified ecosystems is clearly indicated. This theme is returned to later, particularly in chapters 10-13, in which the role of small mammals in arctic, temperate, tropical, and eurasian desert systems is reviewed. It is also referred to in chapter 14, in which control measures for small mammals which are either agricultural pests or disease vectors are stressed.

To study the status and role of a small mammal population within any ecosystem it is not only necessary to have a clear idea of the aims of the study but also some idea of the methods to be used in achieving the objective. The second part of the introduction provides a theoretical treatment of productivity investigations and as such provides a useful background to chapters 2-9. Early chapters cover aspects such as population density estimation and methods of age determination. Demographic patterns are reviewed on a worldwide scale and the role of dispersal in population regulation is discussed. Chapter 6 provides evidence of the value of morpho-physiological indices as predictors of the present and future state of population processes, particularly those concerned with population fluctuations. The three fol-

Birth of a membrane

Membrane Biogenesis: Mitochondria, Chloroplasts and Bacteria. Edited by Alexander Tzagoloff. Pp. xvi+460. (Plenum: New York and London, 1975.) \$27.

THIS collection of articles attempts to review the present state of knowledge and direction of research concerning the mechanisms by which biological membranes are assembled. The various contributors are all expert in their field, and give accounts largely of their own work with only sufficient review material as might be necessary to provide perspective.

About 40% of the book is concerned with yeast or *Neurospora* mitochondria, in articles from the laboratories of H. R. Mahler, A. Goffeau, A. W. Linnane, H. W. Weiss and R. A. Butow. The level of investigation described ranges from the more molecular (Weiss on cytochromes) to the more biological, and of the latter class most are concerned with nuclear-cytoplasmic relationships rather than membrane synthesis *per se*. How far the more biological levels of research will proceed towards a deeper understanding of membrane biogenesis rather

than of mitochondrial genetics is not yet clear, and several of the articles would not be out of place in a volume that was dedicated to some subject quite different from membrane biogenesis. Nevertheless these excellent articles are representative of the field as it is and serve to illustrate the features of control and location that complicate the mechanistic aspects of membrane biogenesis in the mitochondria of eukaryotic cells. It is probably these very complexities which serve to divert workers on eukaryotic cells from some of the central problems of membrane biogenesis, well identified in Tzagoloff's introductory chapter. The same concern with nuclear-cytoplasmic relationships arises in the articles by L. Bogorad, R. J. Ellis and I. Ohad on the biogenesis of chloroplast membranes, which account for 33% of the book.

The remaining 27% of the book consists of articles by M. Inouye, L. Minditch and M. Bayer respectively on the outer, inner and outer-plus-inner cell membranes of *Escherichia coli*. The article by Inouye shows well the penetrating knowledge that can be acquired using molecular and biological approaches with *E. coli*: it could serve as compulsory reading for all who wish to work on membrane biogenesis, for it leads the way in setting up (and achieving) the sort of detailed mechanistic objectives necessary for an understanding of how cells assemble their membranes. Although the outer membrane of *E. coli* may not be very representative of more metabolically active membranes, the inner or cytoplasmic membrane certainly is. Yet there is only one article on the biogenesis of the cytoplasmic membranes of bacteria, and it deals largely with glycerol auxotrophs. The lack of further articles dealing in depth with the use of unsaturated fatty acid auxotrophs of *E. coli* is a notable omission. Also lacking is an account of membrane reconstitution, because a full description of membrane biogenesis will not be claimed until we have a cell-free reconstituted system that makes (or more likely, extends) membranes.

This book should recommend itself mainly to those who research on mitochondria and chloroplasts, but less so in view of its overall emphasis to those who work on bacteria. Teachers of advanced undergraduate courses should also find the book useful for reading selected topics by themselves and their students. As collections of specialist articles go, this book is a good one. Its faults are the usual ones—early obsolescence, and lack of overall balance. Its merits are the excellence and timeliness of the individual articles.

P. B. GARLAND

Fossil plants in palaeoecology

Paleoecology of Terrestrial Plants: Basic Principles and Techniques. By V. A. Krasilov. Pp. viii+283. (Wiley: New York and London; Israel Program for Scientific Translations: Jerusalem, October 1975.) £11.00.

PALAEOECOLOGY is primarily concerned with the reconstruction of past ecosystems from palaeontological and lithological evidence. It is a vast subject that is practiced by scientists of varying disciplines, including geology, botany, zoology, and archaeology. This book fills an important gap in the palaeoecological literature, as it is the first text devoted to the use of fossil plants in palaeoecology.

After a brief introduction in which basic concepts of ecology and palaeoecology are considered, the book is divided into three major parts. The first deals with the burial, transport, and fossilisation of plant material in a variety of sedimentary environments. The second part considers the reconstruction of plant morphology from fossil remains, the environmental deductions that can be made from morphology, and the evolution of plant form. The third part discusses the reconstruction of past vegetation and environment from both fossil pollen and macrofossil remains using a variety of qualitative and quantitative approaches.

These three parts are comprehensive and thorough, with numerous well-chosen examples drawn from a very broad spectrum of both the pre-Quaternary and Quaternary literature. Much of the information is critically assembled and evaluated, although some of the views are unsubstantiated and, in places, are erroneous. For example, the widely held belief (page 144) that the *Taxodium-Nyssa* communities of the south-east US are Tertiary relics has been shown by pollen analytical work in Florida by W. A. Watts to be wrong.

My major criticism of the book is the excessive and largely unjustified use of unusual and often bizarre technical jargon. Terms such as polymeric ecological hyperspace, anthracophobic communities, palynocoenum, xylocenosis, rhizocenosis, selectogenesis, geitogenesis, endoecogenesis, and cenophyllum make reading both difficult and tiring.

In spite of this criticism, the book can be strongly recommended to all botanically-orientated palaeoecologists whether of Quaternary or pre-Quaternary interests who wish to learn more about the basic principles of their subject.

H. J. B. BIRKS